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14. ABSTRACT

Even though 80% of ovarian cancer patients will achieve a complete remission with a combination of surgery and chemotherapy, amost all will recur due to the development of chemoresistance. The WNT/beta-catenin pathway is involved in ovarian cancer growth and suppressing the ability of the immune system to fight off the cancer. Many ovarian cancers have a deficiency in the ability to repair its own DNA, called DNA repair deficiency. Recent clinical efforts have focused on using immune-directed therapies for the treatment of cancer, and specifically ovarian cancer. Although, only a small subset of ovarian cancer patients respond to immunotherapy. Our goal to to gain a better understanding of how DNA repair deficiency and upregulation of the WNT/beta-catenin pathway effect immune response and patient outcomes in ovarian cancer. Our preliminary data show that using a WNT inhibitor for the treatment of ovarian cancer in a mouse model improves the immune system's ability to fight off the cancer. Our central hypothesis is that inhibition of the WNT/beta-catenin signaling pathway will promote antitumor immune response and repress tumor growth, thereby improving clinical response. WNT/beta-catenin genes regulate cell proliferation, thereby mediating cancer initiation and progression and we have successfully targeted this pathway in cells isolated from patients with ovarian cancer and shown that a WNT inhibitor downregulates the WNT pathway and, in a subset of patient samples, caused cell kill. This project will examine the inhibition of the WNT/beta-catenin pathway using a mouse model of spontaneously developing ovarian cancer. In addition, we will also implant ovarian cancer cells with and with out DNA repair deficiency into mice that have an intact immune system. The specific aims of this project are: 1) To determine the relationship between WNT/beta-catenin signaling, the DNA repair pathway, T cell responses and clinical outcomes in ovarian cancer. 2) To determine how inactivation of the WNT pathway by the tumor and dendritic immune cells impacts T cell responses and tumor growth using mouse models. 3) To determine whether mutations that affect DNA repair impact T cell responses following treatment with WNT inhibitors. The proposed research is significant because it will investigate the role of WNT-mediated T cell exclusion from the tumor and elucidate the therapeutic potential of novel WNT inhibitors against ovarian cancer cells with and without a mutation that causes deficiency in the DNA damage repair mechanism.

15. SUBJECT TERMS

None listed

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1. **INTRODUCTION:** Ovarian cancer is the fifth leading cause of death in women in the USA and the most lethal gynecologic malignancy. Given the poor overall survival, high recurrence rates, and rapid development of resistance to chemotherapy in HGSOC, we urgently require new methods to treat this disease. In addition to classic prognostic factors like stage and debulking status, mutations in *BRCA1* and *BRCA2*, components of the homologous recombination (HR) DNA repair pathway, are linked to long-term prognosis in HGSOC. *BRCA1/2* mutations are found in ~20% of HGSOC tumors and are associated with improved prognosis. Interestingly, most BRCA-deficient tumors have activated tumor-infiltrating lymphocytes (TILs), which are also linked to improved outcomes. In contrast, immunosuppressive T regulatory cells (Tregs) and tumor-associated macrophages (TAMs), correlate with a worse prognosis. Despite the link between TILs and positive clinical outcomes, the use of immune checkpoint inhibitors (ICIs) in HGSOC has been disappointing in part due to the "cold" immune landscape surrounding these tumors. A "cold" immune landscape is characterized by the lack of TILs, which sensitize tumors to ICI. Thus, in order to develop better treatments for HGSOC, we need to understand the mechanisms that regulate anti-tumor immune responses, including the role of BRCA1/2 and the HR DNA repair pathway. WNT/β-catenin signaling is also linked to the progression of HGSOC by directly triggering tumor growth (1, 23), and promoting resistance to platinum agents. Interestingly, melanoma-intrinsic activation of the WNT pathway leads to the exclusion of TILs and immune escape by repressing local chemokine expression. In addition, WNT/β-catenin signaling in DCs triggers the expression of immunosuppressive molecules like IL- 10, TGFβ and RALDH, which in turn promote the differentiation of Tregs. Given that infiltrating Tregs and TAMs correlate with a worse prognosis in HGSOC, and that these cells are associated with WNT/β-catenin signaling in other tumors, it is likely that WNT signaling in ovarian cancer also leads to immune suppression. In fact, expression data from 8890 tumor samples (including HGSOC) in The Cancer Genome Atlas (TCGA), show that activating mutations in the WNT/β-catenin pathway, such as *CTNNB1*, encoding β-catenin, and inactivating mutations in negative regulators, such as Axin1, Axin2, APC1, and APC2, are inversely related to inflammatory T cell gene expression signatures. Thus, the purpose of our research is to investigate the inhibition of WNT/β-catenin signaling and its effects on tumor growth and anti-tumor immune responses in ovarian cancer patients with an intact or altered HR DNA repair pathway.

2. **KEYWORDS:** Ovarian cancer, murine ovarian cancer, Wnt, β-catenin, Wnt inhibition, tumor microenvironment, ID8 cells, ID8p53$^{-/-}$ cells, PORCN, WNT-974, CGX-132, DKK1, DKN-01

3. **ACCOMPLISHMENTS:**

   - **What were the major goals of the project?**

	Timeline (Months)	% of Completion
Specific Aim 1		
Major Task 1: Obtain RNA sequencing results from 917 patients from GOG 218	2	25
Major Task 2: Categorize the patients into T cell inflammation subtype, WNT pathway score, HRD status, correlate with survival stratified by treatment	5	25
Milestone(s) Proposed: (1) RNA sequencing data downloaded for 917 patients with HGSOC (2) The relationship between WNT signaling, DNA repair pathway, T cell inflammation subtype and clinical outcomes in HGSC		
Specific Aim 2		
Major Task 1: Determine whether inhibition or activation of the WNT pathway impacts T cell response	9	25
Major Task 2: Create a CRISPR/Cas9-mediated β-catenin knockout model and access T cell response	12	15
Milestone(s) Proposed: (1) Identify the T cell response and tumor response in mice treated with a T cell inhibitor and T cell activator (2) Validate the role of β-catenin in T cell and dendritic cell response		

Specific Aim 3		
Major Task 1: Evaluate the number and types of T cells that are present after treating BRCA2 mutated and wild-type cells	32	25
Milestone(s) Proposed: Determine the difference between immune response to WNT inhibition and activation in cells with and without HR DNA repair deficiency		

- **What was accomplished under these goals?**

Specific Aim 1

Major Task 1: Obtain RNA sequencing results from 917 patients from GOG 218

Subtask 1: Identify location of data

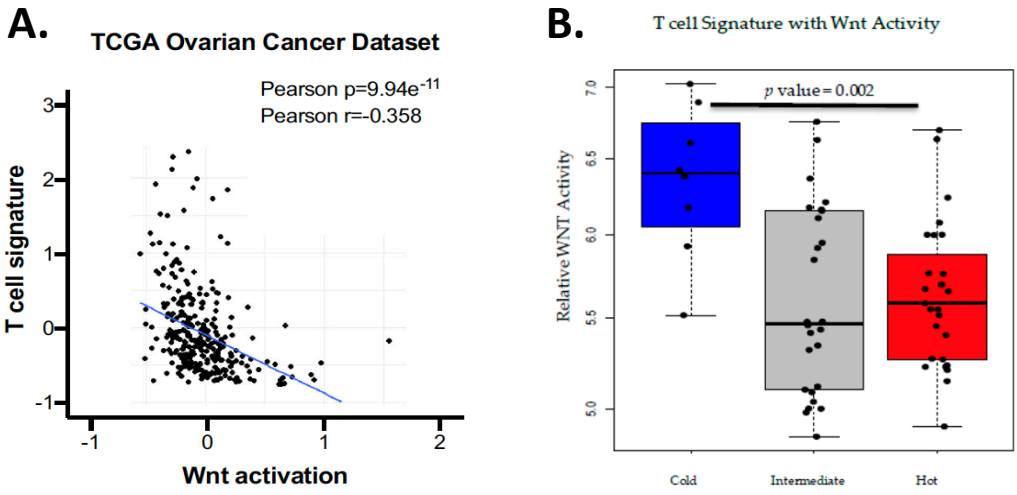
Subtask 2: Download data in a format that can be transferred to Hudson Alpha Institute for analysis

Goals not met: As we were unable to secure data from GOG218 trial, we had to alter our retrieval of data and used The Cancer Genome Atlas (TCGA) project which was a secure, public platform where we were able to retrieve data without hindrance.

Major Task 2: Categorize the patient data into T cell inflammation subtype, WNT pathway score, HRD status, correlate with survival stratified by treatment

Subtask 1: Create categories based on the ovarian TCGA analysis of "hot" and "cold" tumors

When analyzed ovarian cancer samples from the TCGA dataset, we found an inverse correlation between a 13-gene T cell signature and Wnt pathway activating mutations (LRP5 LPR6, FRZ (a family of receptors), DVL, GSK3B, APC, APC2, AXIN (1 and 2), CTNNB1, LEF1, TCF7) (Pearson r= −0.358, p<0.001) (**Figure A**). In addition, we found a negative correlation between Wnt pathway activity and T cell signatures in 57 treatment naïve human high grade serous ovarian cancer (HGSOC) tissue from UAB. Using RNA-seq data to calculate relative Wnt activity and T cell scores (**Figure B**). ***Conclusion:*** **Wnt activating mutations correlated with a decreased "hot" T cell signature.**

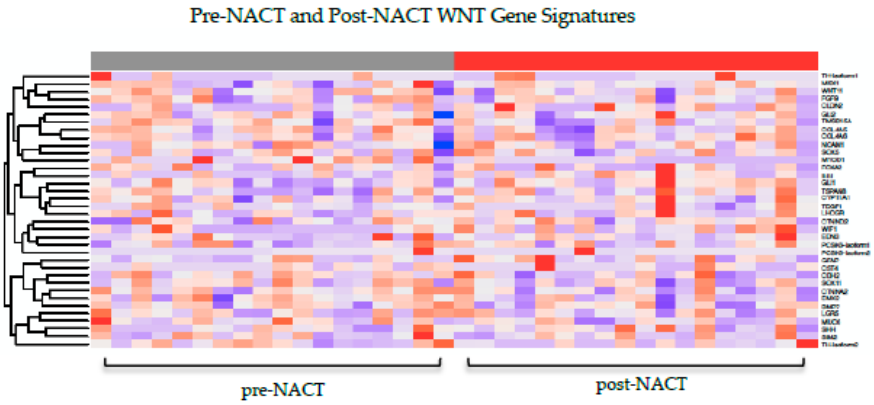


Subtask 2: Analyze the previously collected data (BROCA-HR assay) as it relates to the "hot" and "cold" signature

Subtask 3: Analyze the RNA sequencing data pertinent to HRD status

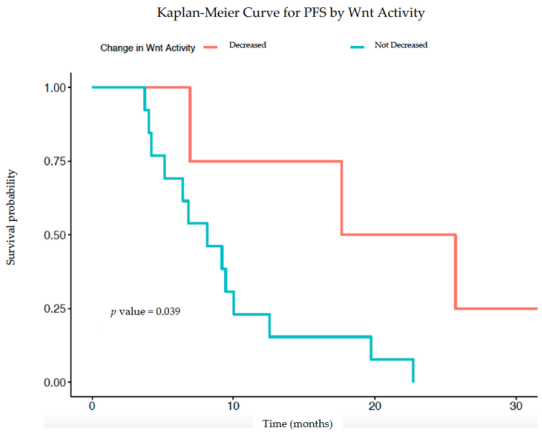
Subtask 4: Categorize patient data based on WNT pathway gene expression

A subset (n = 17) of 57 treatment naïve high grade ovarian cancer samples had matched samples after receiving neo-adjuvant chemotherapy (NACT). Changes in Wnt activity were calculated for these samples and displayed in heatmap below. ***Conclusion: Differential Wnt-related gene expression occurred in responses to NACT.***

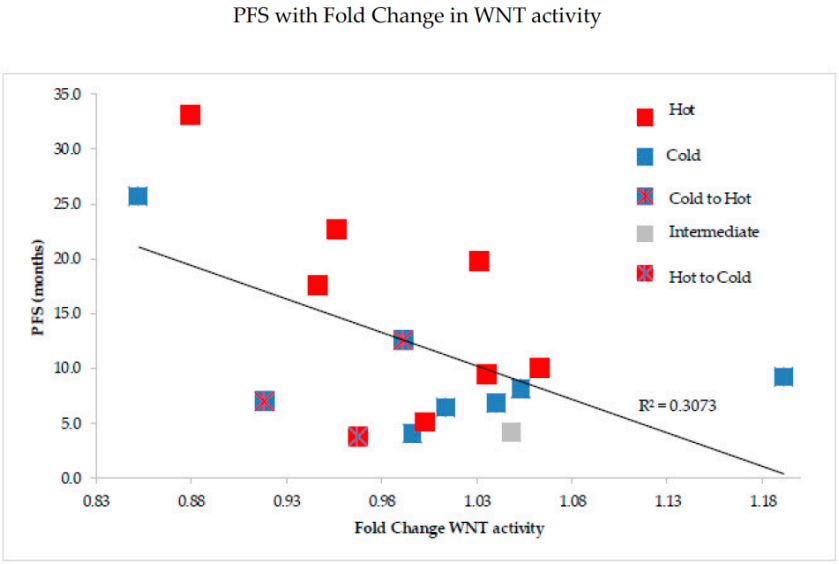


Subtask 5: Combine the data collected in subtask 1-4 and correlate to patient outcomes and treatment

Kaplan-Meier curve for progression-free survival (PFS) (n=17) based on decreases in Wnt activity after neoadjuvant chemotherapy (NACT). ***Conclusion: High Wnt signaling activity corresponds to decreased ovarian cancer patient PFS.***



Patients are labeled based on T cell signature change, with solid color indicating no change, blue square with red lines indicating cold-to-hot signature change, and red square with bluelines indicating a hot-to-cold signature change. ***Conclusion: PFS shows a negative correlation with fold-change in Wnt activity measured by signature genes in matched post- versus pre-NACT in 17 HGSOC patients.***



Goals not met: We were unable to perform analyses with GOG 218 trial data.

Specific Aim 2

Major Task 1: Determine whether inhibition or activation of the WNT pathway impacts T cell response

Subtask 1: Breed enough female Tg*MISIIR-Tag-Low* mice (n=40)

Subtask 2: Inject mice with MOVCAR-luc cells

Subtask 3: Treat mice with vehicle control, WNT974, WNT7A (n=10 in each group) with one untreated group

Subtask 4: Sac 5 mice at day 14 and the remainder on day 28 and quantify tumor-specific CD8 T cells, test whether the T cells are activated, and send whole tumor for RNA seq

Subtask 5: Dissociate fresh tumor tissue and sort-purify cells into CD3/4+ or CD3/8+ T cells

Subtask 6: Extract RNA from sorted cells and use TCR repertoire sequencing

Goals not met: We lost our *TgMISIIR-Tag-Low* colony and were unable to perform specific objectives/subtasks for major task #1.

Major Task 2: Create a CRISPR/Cas9-mediated β-catenin knockout model and access T cell response

Subtask 1: Create MOVCAR knockout cell line

Subtask 2: Compare T cell infiltration, proportion of TAMs, proportion of Tregs, presence of CD103+ dendritic cells in the β-catenin knockout model compared to wild-type

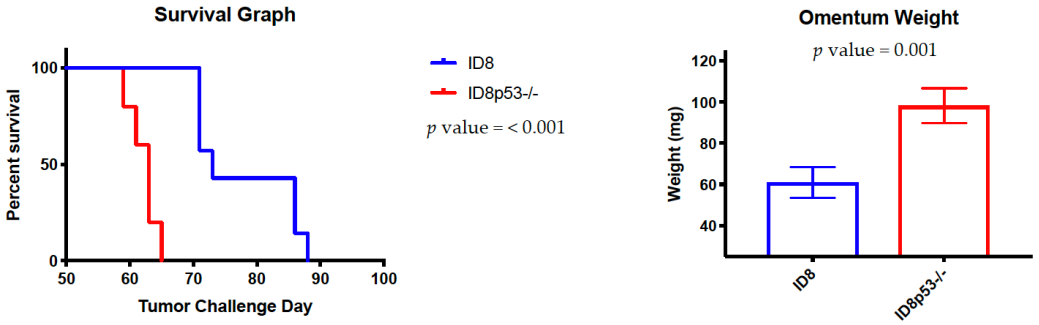
Goals not met: Goals not met: We lost our *TgMISIIR-Tag-Low* colony and were unable to perform specific objectives/subtasks for major task #1.

Specific Aim 3

Major Task 1: Evaluate the number and types of T cells that are present after treating BRCA2 mutated and wild-type cells

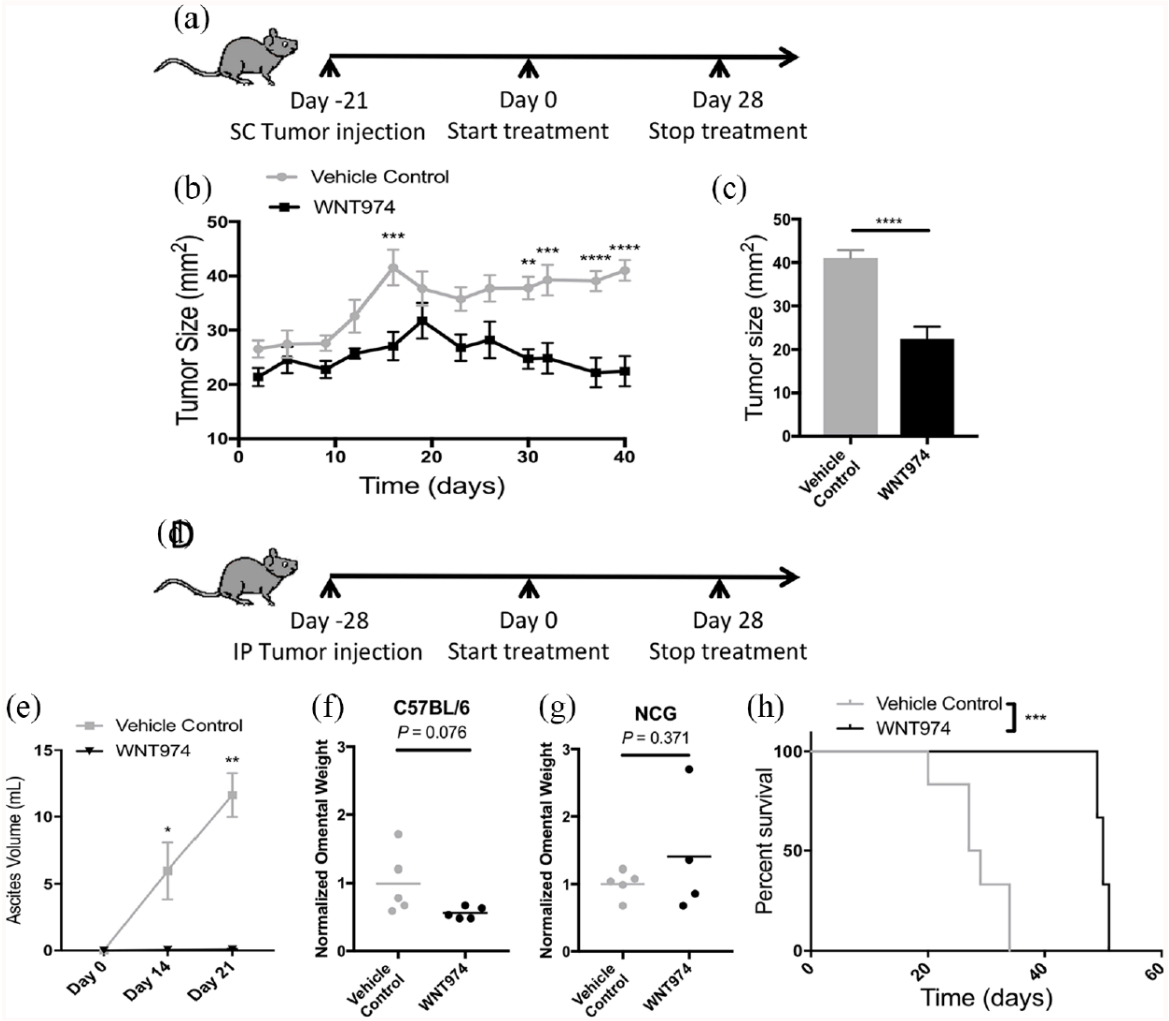
Subtask 1: Implant C57BL/6 mice with ID8 cells that have been modified using CRISPR/Cas9 to lack TRP53 or both TRP53 and BRCA2

Survival was increased with ID8 intraperitoneal tumor challenge in C57Bl/6 mice compared with ID8p53-/- tumor challenge. On tumor challenge day 42, omentum weights between ID8 and ID8p53-/- tumor challenge was statistically different. ***Conclusion: ID8 and ID8p53-/- had differences of survival and tumor burden.***

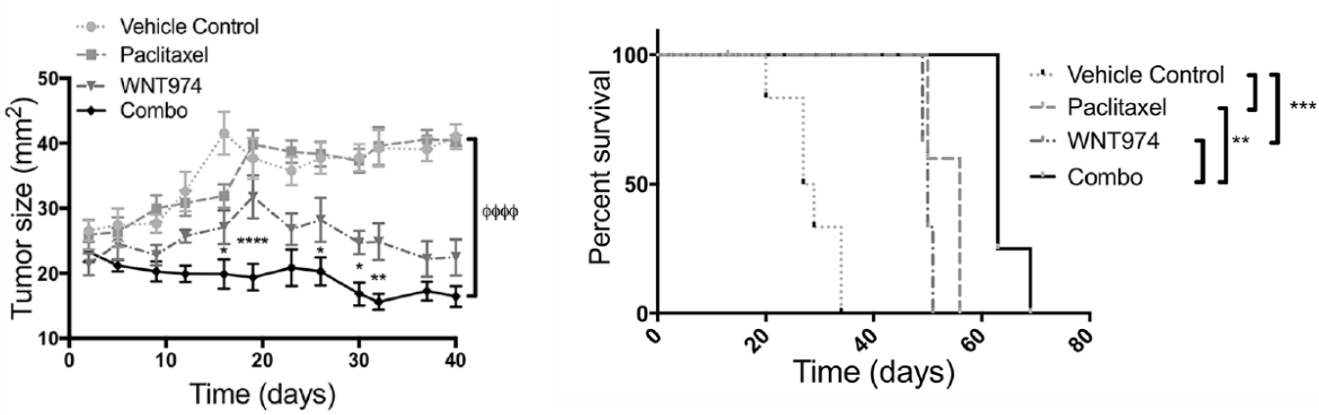


Subtask 2: Treat mice with vehicle control, WNT974, WNT7A (n=10 in each group) with one untreated group

(A) Subcutaneous (SC) experimental design using ID8 parental cells. **(B)** Mice treated with WNT974 had decreased SC tumor growth compared to controls. **(C)** Average SC tumor size at day 40 was significantly smaller in WNT974-treated mice (n = 7 mice/group, data from one of two independent experiments). **(D)** Intraperitoneal (IP) experimental design. **(E)** WNT974-treated mice with IP tumors had fewer ascites than control mice (n = 5–7 mice/group, data from one of three independent experiments). **(F)** C57BL/6 mice treated with WNT974 had lower omental weights than control mice (n = 5 mice/group). **(G)** NCG mice treated with WNT974 had similar omental weights to control mice (n = 5 mice/group). **(H)** WNT974-treated mice with IP tumors had prolonged survival compared to control mice (n = 6 mice/group). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. ***Conclusion: Wnt inhibition reduces tumor burden and ascites formation and prolongs survival in vivo.***

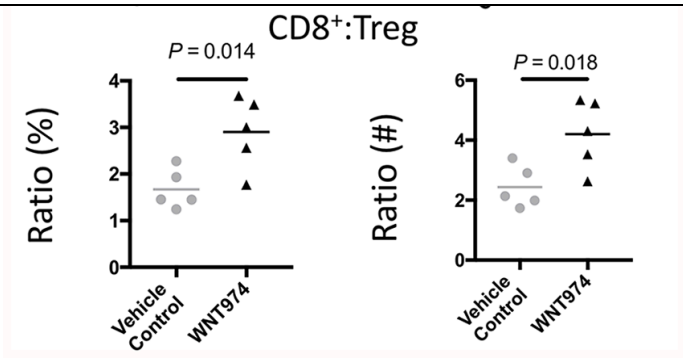


Subcutaneous (SC) tumor growth of ID8 parental cells (n = 7 mice/group, data from one of two independent experiments). * indicates significance between WNT974 and Combo groups. φ indicates significance between vehicle control/paclitaxel and combo groups at endpoint. Treatment was initiated 21 days after SC tumor implantation. Treatment was initiated 28 days after intraperitoneal (IP) tumor injection. Survival curves of mice (n = 6 mice/group). ***Conclusion: Combination of paclitaxel with Wnt inhibition resulted in great reduction in tumor size and enhanced survival.***

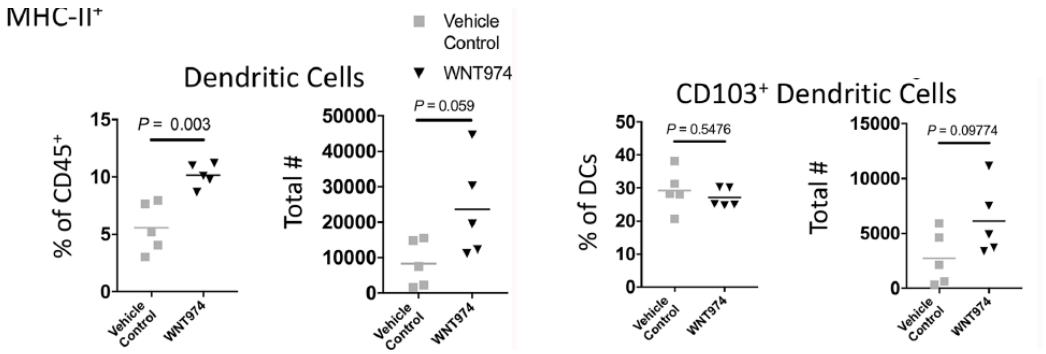


Subtask 3: Quantify and sort CD8+ T cells, CD4+ T cells, and dendritic cells

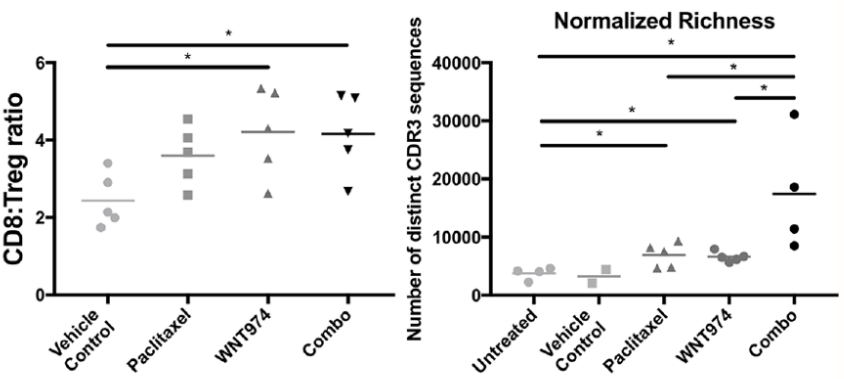
Immune population CD8+ T cell and Treg populations were measured by flow cytometry in omental tumor with or without treatment with WNT-974 for 14 days in C57BL/6 mice with subcutaneous injection of ID8 parental cells. ***Conclusion: Wnt inhibition via WNT-974 significantly increased CD8+ T cell and regulatory T cell (Treg) ratio in the omental tumor of murine ovarian cancer model.***



MHC-II+CD11c^{hi} DC populations measured by flow cytometry in tumor-naive or tumor-bearing mice (ID8 parental cells injected into C57BL/6 mice) with treatment with vehicle control or WNT-974 for 14 days. Percentage frequency of parent shown on the left, total number of cells shown on the right (n = 5–7 mice/group, data from one of three independent experiments, dots represent individual mice). ***Conclusion: Wnt inhibition enhances anti-tumor DC function.***

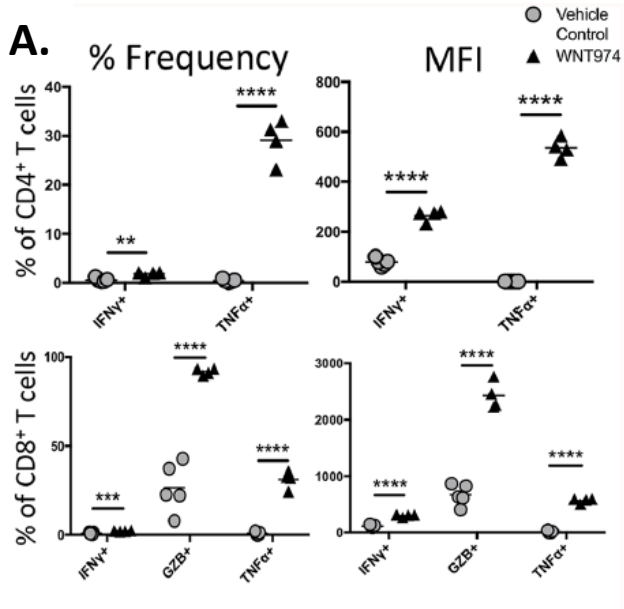


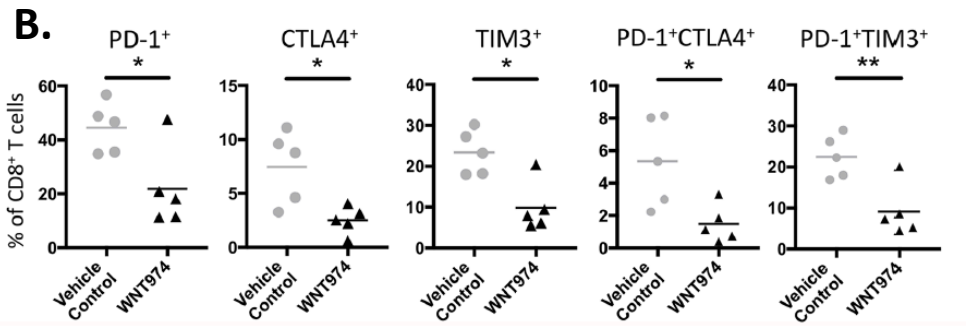
Subcutaneous (SC) tumor growth of ID8 parental cells (n = 7 mice/group, data from one of two independent experiments). Treatment was initiated 21 days after SC tumor implantation. CD8+ T cell:Treg ratio (measured by flow cytometry) and β TCR repertoire analysis of omental tumors after 14-26 days of treatment. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. ***Conclusion: Combination of WNT-974 and paclitaxel results in the greatest increase in CD8+ T cell:Treg ratio and number of distinct CDR3 sequences (β TCR repertoire).***



Subtask 4: Monitor T-cell function

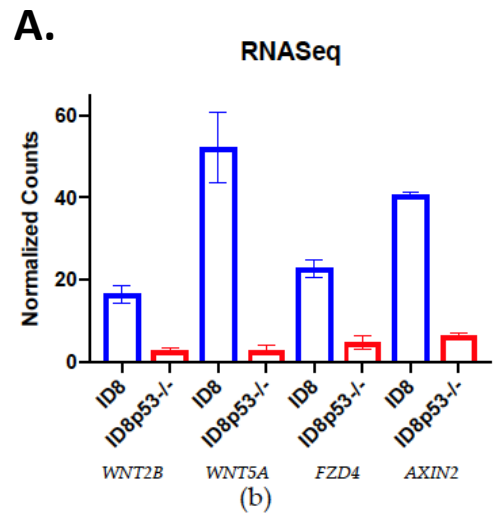
ID8 parental cells were challenged into C57BL/6 mice, and cytokine and inhibitory marker expression from omental tumor TILs after 14 days of treatment was analyzed by flow cytometry. CD4+ T cells (**Figure A - top**) and CD8+ T cells (**Figure A - bottom**), percentage frequency (left) and mean fluorescence intensity (MFI) (right) (n = 5 mice/group, data from one of two independent experiments). Percentage frequency of CD8+ T cells expressing inhibitory markers (**Figure B**) (n = 5 mice/group, data from one of two independent experiments, dots represent individual mice). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. ***Conclusion: Wnt inhibition enhances anti-tumor immune response and reduces immune cell exhaustion.***

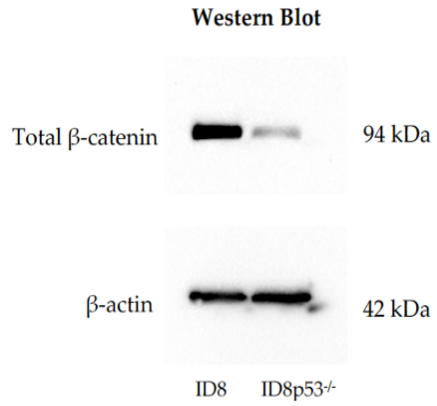




Subtask 5: Monitor gene expression by RNA seq (WNT, immune, HRD signatures)

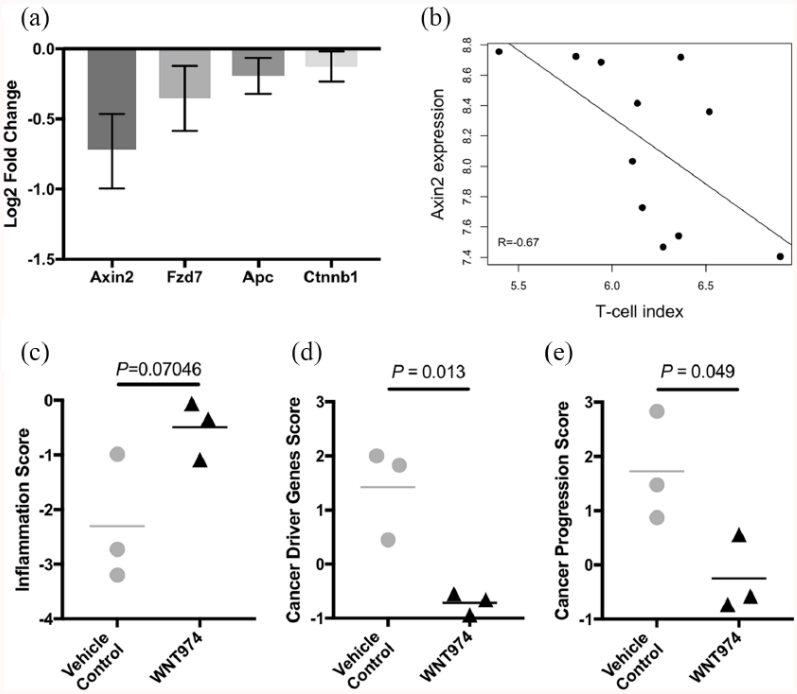
Characterization of Wnt profile in ID8 and ID8p53$^{-/-}$ cell lines. Normalized Wnt related gene expression data for WNT2B, WNT5A, FZD4, and AXIN2 in ID8 (n=6) and ID8p53$^{-/-}$ (n=6) was decreased in the ID8p53$^{-/-}$ cell line compared to the parental ID8 cell line (**Figure A**). Western blot analysis revealed reduced beta-catenin levels in the ID8p53$^{-/-}$ cell line comparted to ID8 cell line (**Figure B**). ***Conclusion: p53 mutation results in decreased Wnt activity in ID8 model.***



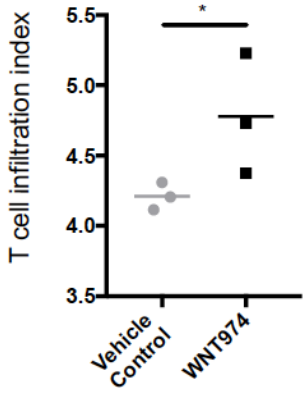


mRNA expression patterns from omental tumors developed from C57BL/6 mice challenged with ID8 cells with 14 days of WNT-974 versus vehicle control. **(A)** Log2 fold change of target genes in the Wnt pathway after treatment with WNT974. **(B)** Gene expression of Axin2 negatively correlates with T cell infiltration. **(C)**

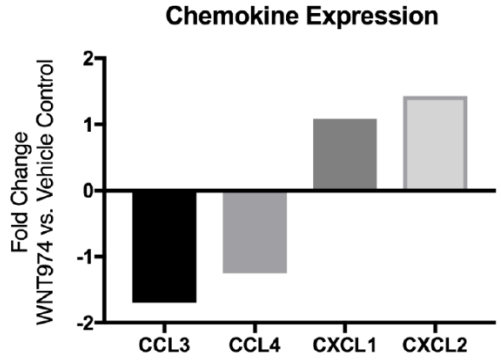
NanoString-defined inflammation score. **(D)** NanoString-defined cancer driver genes score. **(E)** NanoString-defined cancer progression score. n = 3 mice/group for each panel. ***Conclusion: Wnt inhibition promotes an anti-tumor gene expression pattern.***



Excised SC tumors from C57BL/6 mice challenged with ID8 cells were analyzed for mRNA expression. Statistical significance was determined using the t-test. T cell infiltration signature was calculated (n=3/group, 2 at day 7, 1 at day 14). * $P < 0.05$. ***Conclusion: A gene signature of T cell infiltration is up-regulated in the WNT-974 treatment arm compared to control.***



Expression of chemokines by omental tumors in C57BL/6 mice challenged with ID8 cells with WNT-974 and vehicle treatment, as measured by Nanostring. ***Conclusion: Wnt inhibition increased CXCL1 and CXCL2 expression while decreasing CCL3 and CCL4 expression. CXCL1 is responsible for recruitment various inflammatory immune cells and CXCL2 promotes inflammatory response.***



Subtask 6: Extract RNA and use the iRep technique
Goals not met: We were unable to treat mice with WNT7A ligand. HRD gene signatures still need to be analyzed from isolated RNA, as well as iRep technique. In addition, we have not yet gotten to the point of using *BRCA*-mutated murine model.

- **What opportunities for training and professional development has the project provided?**

  I completed a Master of Science in Public Health through the School of Public Health in Clinical and Translational Sciences. The program allowed for the development of skills required for clinical research and academic medicine including clinical trial design, ethics, informatics, biostatistics, grant writing, and data collection and management. Through the program I was able to network with mentors and advisors that she will maintain relationships with throughout her career.

  As faculty at UAB, I have had many opportunities to serve both on the institutional and national level. My interest in personalized medicine has led me to a few different roles here at UAB as I have developed the Personalized Medicine project within the Gyn Onc clinic, in which we have the ability to run NGS on patient tumor samples to understand how these results can affect their standard of care. At present, I have acted as the alternate chair for the Personalized Medicine Working Group since 2017. Another opportunity to use this interest has been serving as a member on UAB's Molecular Tumor Board, where I get the chance to review complex cases and make recommendations based on the patient's molecular profile. In addition to these groups, I am currently serving as a member on the Search Committee for Preventative Medicine. I have served as the chair of the Tissue Committee since 2015, and I have continued to be the chair of UAB's Tissue Committee and to optimize a pathway for tissue banking of ovarian cancer patients and other cancer patients at UAB O'Neal Comprehensive Cancer Center. As chair of the Tissue Committee, I work with a multidisciplinary group of people to see through that human tissue collection for basic and translational research is running as smoothly and efficiently as possible to ensure both patient safety and quality research. I have continued to serve at the co-chair of the Gynecologic Oncology Disease Oriented Working Group (DOWG) and the Precision Oncology Working Group (POWG) where I have enhanced my involvement in the scientific and ovarian cancer research communities at UAB. My responsibilities in these groups include reviewing trials for feasibility and assuring that clinical trials in the pipeline are moving efficiently to opening for enrollment. I am also an active member of the Protocol Review Committee as well as three Clinical Trials Committees: Gyn, Breast, and Phase 1. Lastly, I serve as the Project Leader for the Cervical SPORE Biorepository at UAB.

  I also serve several different roles in national groups outside of the university. Particularly, I am very active in the Society of Gynecologic Oncology (SGO), NRG Oncology (formerly the Gynecologic Oncology Group (GOG)), as well as the American Association for Cancer Research (AACR). I am currently the co-chair of Translational Science on the NRG Ovarian Committee as well as the Ovarian Committee representative to the NRG Translational Research Committee. For the SGO, I have served for two years now as the Emerging Clinical Trialists Course Co-Director. I have also had the honor of serving on the SGO Program Committee and Steering Committee, as well as the SGO Research and Awards committee. Additionally, I was selected to be the on the program committee for SGO 2020. Lastly, my other national service activities have been as co-chair of the FDA-AACR-SGO Workshop, as well as a previous member on the NCI Research Taskforce in 2019.

  I have had the opportunity to collaborate with many scientists both at UAB and around the country by providing samples from my lab, performing key experiments, and supplying my expertise in gynecologic cancers. In addition to the many publications I have served as mentor or collaborator on since joining faculty at UAB, I have also been first author several publications including both reviews and scientific papers (PMIDs 29523763, 30139839, 29843906, 29455465, and 31320488). My foundation in translational research has led me to serve as Co-Investigator or Investigator on several national trials. Those that I am currently serving on are as follows:

1. A Phase 2 Study Evaluating the Efficacy and Safety of DKN-01 as a Monotherapy or in Combination with Paclitaxel in Patients With Recurrent Epithelial Endometrial or Epithelial Ovarian Cancer (**UAB 17105**, NCT03395080). Principal Investigator.
2. Pilot Study of Daily Exemestane in Women with Complex Atypical Hyperplasia of the Endometrium / Endometrial Intraepithelial Neoplasia or Low Grade Endometrial Cancer (**UAB 1788**, NCT03300557). Sub-Investigator.
3. A Phase 1 Trial of M4344 and Niraparib in patients with PARP resistant recurrent ovarian cancer (**UAB 1885**, NCT04149145). Principal Investigator.
4. Cabozantinib Plus Nivolumab and Ipilimumab Women With Recurrent Gynecologic Carcinosarcoma (**UAB 1921**, NCT04149275). Principal Investigator.
5. A Phase 2, Single Arm Study of Atezolizumab + Bevacizumab in Women with Advanced, Recurrent or Persistent Endometrial Cancer (**UAB 18107**, NCT 03526432). Co-Investigator.
6. Phase 1 trial of CB-839 in Combination with Niraparib in platinum resistant BRCA-wild type Ovarian Cancer Patients (**UAB 1801**, NCT03944902). Co-Investigator.

NCT03395080 is a study investigating the Wnt modulator DKN-01 (Leap Therapeutics) alone or in combination with paclitaxel in women with recurrent endometrial and epithelial ovarian cancer. Recently the trial has been expanded to include an arm for women with recurrent carcinosarcomas due to promising responses seen previously in these patients. Two current trials (both Investigator Initiated Trials) include novel therapies for PARP inhibitor resistant ovarian cancer patients – one using the ATR inhibitor M4344 (NCT04149145) and the other using the glutaminase inhibitor CB-839 (NCT03944902). NCT04149275, treatment with cabozantinib along with ipilimumab/nivolumab for gynecologic carcinosarcomas is also an Investigator Initiated Trial.

Dr. Birrer, Dr. Odunsi, and myself had combined lab meetings and bi-montly one-on-one meetings to discuss her career development and research progress. In addition, I had weekly lab meetings with Dr. Sara Cooper from HudsonAlpha and Dr. Troy Randall from UAB to discuss on-going projects. My lab members and myself went to both Dr. Birrer and Dr. Randall's labs to learn molecular biology and immunology techniques that were not previously known.

I trained the gynecologic oncology clinical fellows Drs. Whitney Goldsberry and David Doo on this grant. In addition to these fellows, I have also directly and indirectly mentored several PhD, MD, and MD/PhD students. They had the opportunity to perform basic science research, exploring the role of the Wnt signaling pathway in progression of ovarian cancer. In addition, they each wrote a review and research article pertaining to their projects. They were required to present their findings at weekly lab meetings and once each semester to the Department of OB/GYN – Gynecologic Oncology Division. Furthermore, they each presented their research projects with either a poster or oral presentation at SGO. I was able to attend the AACR general meeting, ASCO meeting, and SGO meeting. These meetings allowed for me to gain further knowledge and expertise in ovarian cancer and the immune system.

- **How were the results disseminated to communities of interest?**

  The results from these studies were presented at local and national conferences to audiences that are in similar areas of research and/or medicine.

- **What do you plan to do during the next reporting period to accomplish the goals?**

Specific Aim 1
Major Task 1: Obtain RNA sequencing results from 917 patients from GOG 218
Subtask 2: Obtain RNA sequencing results from 917 HAS data from GOG 218
Subtask 3: Download data in a format that can be transferred to Hudson Alpha Institute for analysis
Proposed experiments: We are awaiting data from the GOG 218 trial, so as to perform the same analyses as in Major Task #2.
Major Task 2: Categorize the patient data into T cell inflammation subtype, WNT pathway score, HRD status, correlate with survival stratified by treatment
Subtask 1: Create categories based on the ovarian TCGA analysis of "hot" and "cold" tumors

Subtask 2: Analyze the previously collected data (BROCA-HR assay) as it relates to the "hot" and "cold" signature
Subtask 3: Analyze the RNA sequencing data pertinent to HRD status
Subtask 4: Categorize patient data based on WNT pathway gene expression
Proposed Experiments: Analyze the GOG 218 (once acquired) to determine "hot" and "cold" signatures, RNA sequencing data as it related to HRD status, and categorize data based on WNT pathway gene expression. HRD gene expression profiling from our previous analysis of TCGA will be analzed.

Specific Aim 2

Major Task 1: Determine whether inhibition or activation of the WNT pathway impacts T cell response
Subtask 3: Breed enough female *MISIIR-Tag-Low* mice (n=40)
Subtask 4: Inject mice with MOVCAR-luc cells
Subtask 5: Treat mice with vehicle control, WNT974, WNT7A (n=10 in each group) with one untreated group
Subtask 6: Sac 5 mice at day 14 and the remainder on day 28 and quantify tumor-specific CD8 T cells, test whether the T cells are activated, and send whole tumor for RNA seq
Subtask 7: Dissociate fresh tumor tissue and sort-purify cells into CD3/4+ or CD3/8+ T cells
Subtask 8: Extract RNA from sorted cells and use TCR repertoire sequencing
Proposed Experiments: Acquire new mice to breed and expand *TgMISIIR-Tag-Low* mouse colony to perform experiments proposed.
Major Task 2: Create a CRISPR/Cas9-mediated β-catenin knockout model and access T cell response
Subtask 1: Create MOVCAR knockout cell line
Subtask 2: Compare T cell infiltration, proportion of TAMs, proportion of Tregs, presence of CD103+ dendritic cells in the β-catenin knockout model compared to wild-type
Proposed Experiments: Same as above.

Specific Aim 3

Major Task 1: Evaluate the number and types of T cells that are present after treating BRCA2 mutated and wild-type cells
Subtask 1: Implant C57BL/6 mice with ID8 cells that have been modified using CRISPR/Cas9 to lack TRP53 or both TRP53 and BRCA2
Subtask 2: Treat mice with vehicle control, WNT974, WNT7A (n=10 in each group) with one untreated group
Subtask 3: Quantify and sort CD8+ T cells, CD4+ T cells, and dendritic cells
Subtask 4: Monitor T-cell function
Subtask 5: Monitor gene expression by RNA seq (WNT, immune, HRD signatures)
Subtask 6: Extract RNA and use the iRep technique
Proposed Experiments: Perform additional in vivo analyses treating mice with WNT7A and untreated, and perform sequent analyses (subtasks 3-6) proposed with this model.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

  The data obtained from these studies lay the foundation for treatment strategies that will optimize ovarian cancer patient response to immunotherapy.

- **What was the impact on other disciplines?**

  The data obtained from these studies lay the foundation for treatment strategies that could enhance cancer patient immune response, and ultimately lead to improved therapeutic success via increased sensitivity to immunotherapy.

- **What was the impact on technology transfer?**

  Nothing to Report.

- **What was the impact on society beyond science and technology?**

  I continue to serve on multiple boards and panels that pertain to patient education and patient advocacy.

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

  In ***Specific Aim 1***, we were unable to secure data from GOG 218 trial, so we had to alter our retrieval of data and used The Cancer Genome Atlas (TCGA) project, a secure, public platform where we were able to retrieve data without hindrance.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

  During the course of experiment, we lost our *TgMISIIR-Tag-Low* colony at UAB, and because of this we were unable to accomplish part of ***Specific Aim 2***. We are in process of writing the MTA to acquire the mouse strain from Dr. Connolly's lab at Fox Chase Cancer Center.

- **Changes that had a significant impact on expenditures**

  In addition, it came to our attention that the amount of training needed to prepare clinical fellows and graduate students to properly execute proposed experiments was mirrored by a lack in proposed supply utilization (e.g. mice, drugs, etc.). During the time of this progress report period, we had only one research technician to aid in the execution of experiments and utilization of supplies. This resulted in an excess of unused funds that we will use in the next progress report period with the start of a new postdoctoral trainee.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  Nothing to Report.

- **Significant changes in use or care of human subjects.**

  Nothing to Report.

- **Significant changes in use or care of vertebrate animals.**

  MISIIR-Tag colony was lost, but we are the process of acquiring new animals to gain back colony at UAB.

- **Significant changes in use of biohazards and/or select agents.**

  Nothing to Report.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**:

  - **Journal publications.**

    1. Goldsberry, W., *et al.* (June 2019). A review of the role of Wnt in cancer immunomodulation. *Cancers.* 11, 771. DOI: 10.3390/cancers11060771. (No federal support)

2. Doo, D. Norian, L., and Arend, R. (June 2019). Checkpoint inhibitors in ovarian cancer: A review of preclinical data. *Gyn Onc Reports* 29: 48-54. (No federal support)

- **Books or other non-periodical, one-time publications.**

  Nothing to Report.

- **Other publications, conference papers, and presentations.**

1. SGO Annual Meeting – Goldsberry, W., *et. al.* (March 2019). Inhibition of PORCN in a p53-/- Knockout Syngeneic Ovarian Cancer Model. *Abstract.*
2. SGO Annual Meeting – Doo, D. *et al.* (March 2019). Inhibition of the Wnt/β-catenin pathway enhances anti-tumor immunity in ovarian cancer. *Abstract.*

- **Website(s) or other Internet site(s)**

  Nothing to Report.

- **Technologies or techniques**

  Nothing to Report.

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

  Nothing to Report.

- **Other Products**

  - ***Biospecimen collections:***

    During the course of study, we were able to bank multiple ovarian cancer tissues and matching blood from patients at UAB. These specimens will be used in patient-derived xenografts (PDX) and 3-dimensional (3-D) models to further study the effects of Wnt pathway alteration on immune response.

  - ***Clinical interventions:***

    The results from this study provide evidence for the use of Wnt inhibitors as a means to enhance ovarian cancer's anti-tumor immune response. Importantly, these interventions could sensitize ovarian cancer to immune checkpoint blockade (ICB) therapy.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	**Dr. Rebecca Arend**
Project Role:	Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-2108-3426
Nearest person month worked:	3
Contribution to Project:	Dr. Arend has conceptualized and designed all the mouse model experiments. She has given expert advice for

	treatment regimen and harvesting and processing tissues from experiments.
Funding Support:	N/A

Name:	**Dr. Michael Birrer**
Project Role:	Mentor
Researcher Identifier (e.g. ORCID ID):	0000-0001-6464-4225
Nearest person month worked:	1
Contribution to Project:	Dr. Birrer has provided mentorship in my career and guidance in designing experiments and analyzing data. He is also helping in acquisition of data from GOG trials.
Funding Support:	N/A

Name:	**Dr. Kunle Odunsi**
Project Role:	Mentor
Researcher Identifier (e.g. ORCID ID):	0000-0002-4444-7651
Nearest person month worked:	1
Contribution to Project:	Dr. Odunsi has provided guidance in designing experiments, professional guidance and analyzing data.
Funding Support:	N/A

Name:	**Dr. Troy Randall**
Project Role:	Mentor
Researcher Identifier (e.g. ORCID ID):	0000-0003-0643-0311
Nearest person month worked:	1
Contribution to Project:	Dr. Randall provided guidance in experimental design related to immunology.
Funding Support:	N/A

Name:	Dr. Sara Cooper
Project Role:	Faculty Investigator at Hudson Alpha Institute for Biotechnology
Researcher Identifier (e.g. ORCID ID):	0000-0002-9627-0309
Nearest person month worked:	9
Contribution to Project:	Dr. Cooper has analyzed RNA sequencing data for this study.
Funding Support:	N/A

Name:	Dr. David Doo
Project Role:	Clinical Gynecologic Oncology Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	9
Contribution to Project:	Dr. Doo has performed and contributed to *in vitro* and *in vivo* experiments described in the study.
Funding Support:	N/A

Name:	Dr. Whitney Goldsberry
Project Role:	Clinical Gynecologic Oncology Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	9
Contribution to Project:	Dr. Goldsberry has performed and contributed to *in vitro* and *in vivo* experiments described in the study.
Funding Support:	N/A

Name:	Ashwini Katre
Project Role:	Research Assistant
Researcher Identifier	

(e.g. ORCID ID):	
Nearest person month worked:	9
Contribution to Project:	Ashwini Katre has performed animal experiments described in the study.
Funding Support:	N/A

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Dr. Birrer is expected to leave UAB in December 2019, and Dr. Troy Randall has agreed to become on-site mentor in Dr. Birrer's absence. However, Dr. Birrer will remain my mentor and we will continue to have scheduled meetings via zoom.

What other organizations were involved as partners?

Organization Name	**Novartis**
Location of Organization	Basel, Switzerland
Partner's contribution to the project	
Financial support	N/A
In-kind support *(e.g., partner makes software, computers, equipment, etc., available to project staff)*;	Supplied WNT-974 compound for studies
Facilities *(e.g., project staff use the partner's facilities for project activities)*;	N/A
Collaboration *(e.g., partner's staff work with project staff on the project)*;	N/A
Personnel exchanges *(e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)*;	N/A
Other	N/A

Organization Name	**HudsonAlpha Institution for Biotechnology**
Location of Organization	Huntsville, AL
Partner's contribution to the project	
Financial support	N/A
In-kind support *(e.g., partner makes software, computers, equipment, etc., available to project staff)*;	N/A

Facilities *(e.g., project staff use the partner's facilities for project activities)*;	N/A
Collaboration *(e.g., partner's staff work with project staff on the project)*;	Analyze RNA-sequencing data
Personnel exchanges *(e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)*;	N/A
Other	N/A

8. **SPECIAL REPORTING REQUIREMENTS**

   - **COLLABORATIVE AWARDS**

     Nothing to Report.

   - **QUAD CHARTS**

     Nothing to Report.

9. **APPENDICES:**