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14. ABSTRACT
The recent addition of bevacizumab to the treatment of primary ovarian cancer and its FDA approval for this disease follow from the AURELIA and GOG/NRG 218 trials. From 1873 enrolled patients on GOG/NRG 218, it was found that the addition of bevacizumab to chemotherapy and its inclusion in the extended period resulted in a significant improvement in progression free survival (PFS) of 4 months compared to chemotherapy alone, with a hazard ratio of 0.717. The critical questions that must now be answered are 1) what are the molecular markers that predict for response on this landmark ovarian cancer trial? and 2) what are alternative treatment strategies for non-responders? Our central hypothesis is that targeted agents including bevacizumab, chosen based upon the knowledge of the *TP53* mutational status, synergize with chemotherapy and/or the PARP inhibitor olaparib and promote catastrophic tumor cell death. These studies are anticipated to have a significant positive impact on the field by enhancing the design and choice of therapy for ovarian cancer based on *TP53* mutations.

15. SUBJECT TERMS
Ovarian cancer; p53; bevacizumab; chemotherapD

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1. Introduction

The recent addition of bevacizumab to the treatment of primary ovarian cancer and its FDA approval for this disease follow from the AURELIA and GOG/NRG 218 trials. From 1873 enrolled patients on GOG/NRG 218, it was found that the addition of bevacizumab to chemotherapy and its inclusion in the extended period resulted in a significant improvement in progression free survival (PFS) of 4 months compared to chemotherapy alone, with a hazard ratio of 0.717. The critical questions that must now be answered are 1) what are the molecular markers that predict for response on this landmark ovarian cancer trial? and 2) what are alternative treatment strategies for non-responders? Our central ***hypothesis*** is that targeted agents including bevacizumab, chosen based upon the knowledge of the *TP53* mutational status, synergize with chemotherapy and promote catastrophic tumor cell death. The rationale for our hypothesis is that *TP53,* the 'guardian of the genome', is mutated in 95% of all high grade serous ovarian cancers and is considered the fundamental early genomic alteration associated with this disease. However, it is critically important to note that varying types of p53 mutant proteins exist, with different implications for cell cycle regulation and for chemosensitivity. Extensive preclinical data from our team demonstrate that the *TP53* mutations alter the master regulators of cell cycle checkpoints in predictable *yet distinct* ways that can be capitalized upon to overcome resistance to chemotherapy using targeted agents that block compensatory survival pathways. We term this principle "molecularly enhanced chemotherapy." Our specific aims are to 1) in a retrospective study of GOG/NRG 218 clinical samples, determine the functional class of *TP53* mutation for each case and correlate with response; 2) assess the mechanisms of resistance to therapy as defined by outcomes from GOG/NRG 218 and identify alternative molecularly enhanced combinations; and 3) interrogate the function of *TP53* mutations that are *somatic variants of unknown function* (sVUF) to define the best therapy for patients with these mutations. These studies are anticipated to have a significant positive impact on the field by enhancing the design and choice of therapy for ovarian cancer based on specific *TP53* mutations. Moreover, our findings have the potential for immediate clinical application since we are performing retrospective analyses of a positive trial to better define a subgroup of patients that are more likely to respond. We will also prioritize drug choice in Aims 2 and 3 to use agents that have proven safety profiles and efficacy in other cancer types. Hence, our findings can be quickly implemented to provide personalized precision treatment options and improve outcomes for women with ovarian cancer using currently defined and FDA-approved treatments.

2. Keywords

Ovarian cancer; p53; bevacizumab; chemotherapy; curcumin analogues; HO-3867; RNA sequencing; Gynecologic Oncology Group Study 218

3. Accomplishments

What were the major goals of the project?

Project/Grant Period: 09/30/2020-08/31/2023	Target Date (Month(s) relative to start date)	Actual Completion Date or % of Completion
Specific Aim 1: From GOG/NRG 218 clinical samples, determine the functional class of TP53 mutation for each case and correlate with response		
Major Task 1: Categorize cases in GOG/NRG 218 based upon *TP53* status.		
Subtask 1: Submit institution's IRB approval and related material for DoD's HRPO approval	1	03/2020
Subtask 2: Receive HRPO approval or exempt finding before initiating human subjects/HAS related studies.	1	03/2020
Subtask 3: Extract de-identified *TP53* sequencing data from GOG/NRG 281 BROCA sequencing files *To be carried out at Site 2.	1-12	100%
Subtask 4: Bin *TP53* mutations into function categories (LOF, GOF, sVUF, WT)	5-36	90%
Milestone Achieved: Bin *TP53* mutations	36	90%
Major Task 2: Correlate binned *TP53* subclass designations with response, PFS and OS		

Subtask 1: Provide NRG Statistical Office with a table containing de-identified patient identifiers and TP53 functional category (LOF, GOF, sVUF, WT)	36	50%
Subtask 2: Receive a report of the correlation between *TP53* mutational status and outcomes on GOG/NRG 218 *25% to be carried out at Site 2.	9	First report on GOF mutations completed; second report on all mutations pending.
Subtask 3: Assemble data from Major Tasks 1-2 for publication *25% to be carried out at Site 2.	9-12	Projected to be completed in the next 12 months
Milestone Achieved: Determine the correlation of TP53 subclass designations with PFS, OS and response overall and on each arm of GOG/NRG 218	12	Projected to be completed in the next 12 months
Specific Aim 2: Assess the mechanisms of resistance to therapy as defined by outcomes from GOG/NRG 218 and identify alternative molecularly enhanced combinations.		
Major Task 3: Determine the functional impact of GOF mutants.		
Subtask 1: Generate cell models engineered to express all GOF mutants in GOG/NRG 218 dataset	5-36	70% completed
Subtask 2: Assess the impact of each GOF mutant on canonical p53 WT function	8-36	70% completed
Subtask 3: Assess the impact of each GOF mutant on cell cycle checkpoint signaling	8-36	70% completed
Subtask 4: Define the impact of each GOF mutant on the transcriptome using RNAseq	13-36	70% completed
Milestone(s) Achieved: Assign function to each TP53 GOF mutant	16	Projected to be completed in the next 6 months
Major Task 4: Identify the most effective molecular inhibitor to combine with chemotherapy in order to overcome the impact of each GOF mutant		
Subtask 1: Test the impact of select agents on cell viability, cell cycle progression, percent of mitotic cells and activation of cell cycle checkpoint	17-24	75% completed
Milestone Achieved: Identify appropriate molecular agent to use in studies in Major Task 5 (Aim 2C)	24	75% completed
Major Task 5: Determine the *in vivo* efficacy of the best combinations from Aim 2B		
Subtask 1: Obtain local IACUC Approval for proposed studies in PDX mice	1	02/21/2020
Subtask 1: Submission of institution approved animal protocols and related material for DoD's ACURO approval	1	03/06/2020
Subtask 2: Receive ACURO approval before initiating animal experiments.	1	05/2020
Subtask 2: Perform drug testing studies in PDX mice	25-36	100% completed
Subtask 3: Analyze post-treatment tumor specimens from PDX mice	28-36	100% completed
Milestone Achieved: IACUC approval for animal studies	3	100% completed
Milestone Achieved: Assemble final data for Major Tasks 3-5	36	100% completed
Milestone Achieved: Manuscript preparation for data generated in Major Tasks 3-5	36	Manuscript published in *Cell Death & Disease* 01/2022;
Specific Aim 3: Interrogate the function of TP53 mutations that are somatic variants of unknown function to define the best therapy for patients with these mutations		
Major Task 6: Develop a predictive algorithm to bin sVUF in GOG/NRG 218 into functional categories		
Subtask 1: Estimate mutation effect of each sVUF in the GOG/NRG 218 cohort *in silico*	6-36	75% completed
Subtask 2: Predict functional effect of mutations	6-36	75% completed
Subtask 3: Obtain FFPE slides from NRG Oncology for immunohistochemistry (IHC)	36	Projected to be completed in next 12 months
Subtask 4: Perform IHC for p53 protein expression and bin cases	36-48	Projected to be completed in next 12 months
Milestone Achieved: Functionally bin cases as WT, LOF or GOG	36-48	Projected to be

		completed in next 18 months
Major Task 7: Binning based on *in vitro* assessment of mutant p53 activity.		
Subtask 1: Generate cell models engineered to express recurrent sVUFs in GOG/NRG 218 dataset	5-8	75% completed
Subtask 2: Assess the impact of each sVUF mutant on canonical p53 WT function, cell cycle and transcriptome	8-16	50% completed
Milestone(s) Achieved: Assign function to each TP53 sVUF mutant	16	Projected to be completed in 12 months
Major Task 8: Determine the impact of *TP53* sVUF on sensitivity to chemotherapy		
Subtask 1: Test the impact of select agents on cell viability, cell cycle progression, percent of mitotic cells and activation of cell cycle checkpoint	17-24	50% completed
Subtask 2: Drug testing studies in PDX mice	25-36	Completed, published in *Cell Death and Disease*, Bi J et al. 2022
Milestone Achieved: Manuscript preparation for data generated in Major Tasks 6-7	36	Projected to be completed in next 24 months

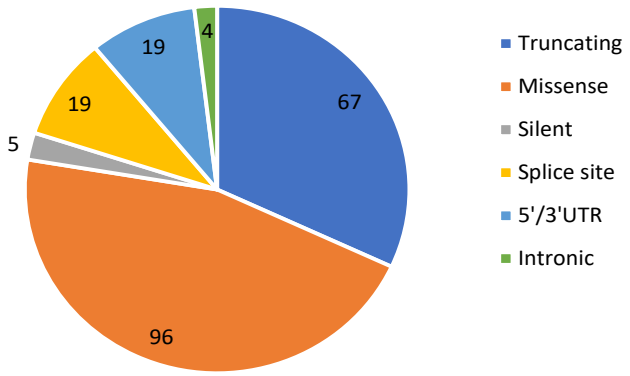
What was accomplished under these goals? Here we detail the accomplishments by Specific Aim and Subaim.

Specific Aim 1 From GOG/NRG 218 clinical samples, determine the functional class of TP53 mutation for each case and correlate with response.

Aim 1A: Categorize cases in GOG/NRG 218 based upon *TP53* status.

- We have received approval from the NRG Oncology Ancillary Projects Committee approval for TR studies.
- Our collaborators at the University of Washington have provided a list of mutations in *TP53* that occurred as single events among the 713 patients with available sequencing data enrolled on Arms 1 and 3 of GOG-218.
- We have binned these cases based upon the specific type of TP53 mutation as in **Figure 1**.
- We now have received the entire list of mutations in TP53 found in the cases from GOG-218 and have begun to study the impact of those mutations on clinical outcomes (**Figure 2**).

Figure 1



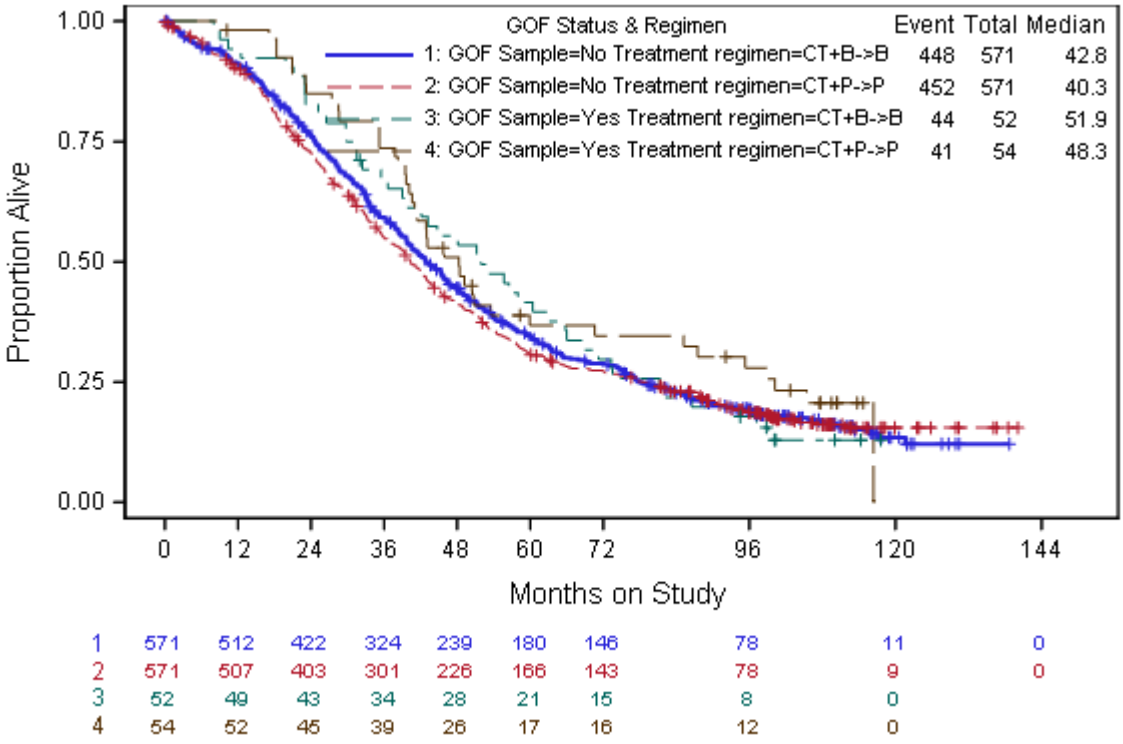


Figure 2. **Overall Survival for patients treated with chemotherapy + bevacizumab versus chemotherapy alone from GOG 218. This sample includes only those TP53 mutations that are categorized as "gain of function" or GOF. There is a trend for improvement in overall survival in those high-risk cases with the addition of bevacizumab in this preliminary analysis; however, additional p53 mutations must be evaluated from this dataset in order to understand the full impact of bevacizumab in addition to chemotherapy in ovarian cancer.**

We are now assessing the entire number of cases in GOG study 218 with p53 missense mutations to increase the number of patients for analysis. <u>We are requesting a no cost extension for the coming year to complete the following critical steps for Aim 1</u>:

We hypothesize that patients who received bevacizumab+chemotherapy with specific mutations in p53 have improved outcomes as compared to patients who received chemotherapy only. Studies utilize existing next-generation sequencing data for p53 for patients from Arm 1 (carboplatin+paclitaxel) and Arm 3 (carboplatin+paclitaxel+bevacizumab with maintenance bevacizumab) of GOG-218, a study of ovarian cancer. The specific goals include:

1. Bin p53 mutations into categories: A total of 743 p53 variants were identified in our study cohort.
   a. <u>Wild-type (WT)</u>: We define cases as WT for p53 if there are no mutations in the protein coding region that change the amino acid sequence. Single nucleotide variants in the 3' or 5' UTR, intronic regions and silent mutations are binned as WT.
   b. <u>Suspected Pathogenic Mutation</u>: We defined suspected pathogenic mutations as any variants that occur in the protein coding region and change the amino acid sequence. This includes missense mutations that result in a single amino acid change, in-frame short deletions or insertions of 1-3 amino acids, nonsense mutations that result in a premature stop codon, insertions/deletions that result in a frameshift, and splice site mutations. After filtering out variants in the 3' and 5' UTR, silent mutations and intronic variants, a total of 595 mutations in p53 were identified across 570 cases.
   c. Suspected Pathogenic Mutations are further subdivided into three functional categories for additional analyses:
      i. <u>Missense</u> and in-frame short insertions and deletions
      ii. <u>Truncating mutations</u>: nonsense, splice and frame-shift insertions/deletions
   d. For cases with more than one variant in the p53 gene, we will bin based on the predominant variant as determine by the variant allele frequency (VAF). We have identified 21 cases with >1 mutation in p53 (46

unique mutations). In subsequent analyses, we will exclude any cases with more than one variant that cannot be confidently binned based on the VAF data.

2. Determine the effect of p53 mutational status on outcomes: treatment-independent effect. We hypothesize that patients with Suspected Pathogenic, Missense or Truncating mutations have worse progression-free survival (PFS) and overall survival (OS) as compared to patients with WT p53, regardless of treatment. We will generate Kaplan-Meier survival curves and determine the hazard ratios for PFS and OS
3. Examine whether addition of bevacizumab to the chemotherapy backbone predicts for better outcomes in cases with mutated p53. We hypothesize that patients with Suspected Pathogenic, Missense or Truncating mutations better worse progression-free survival (PFS) and overall survival (OS) when treated with bevacizumab + chemotherapy vs. chemotherapy alone. We will generate Kaplan-Meier survival curves and determine the hazard ratios for PFS and OS to analyze the treatment effect.
4. Determine the distribution of p53 mutational types by patient characteristics, including tumor histology, grade, and racial/ethnic group.
5. Future studies include acquiring FFPE samples, if available, and performing immunohistochemistry (IHC) for p53 and for other pro-angiogenic biomarkers. These studies will provide important insight into the concordance of p53 mutations with abnormal expression at the protein level, as determined by IHC. We would then determine the ability of p53 status by IHC to predict improved response to bevacizumab-containing chemotherapy for patients with ovarian cancer.

<u>Specific Aim 2: Assess the mechanisms of resistance to therapy as defined by outcomes from GOG/NRG 218 and identify alternative molecularly enhanced combinations.</u>

<u>Aim 2A: Determine the baseline impact of various GOF mutants on cell cycle checkpoint control and expression of canonical p53 transcriptional targets.</u>

- We have generated both cell line models and patient-derived organoid (PDO) models of ovarian cancer harboring various mutations in *TP53*. These data have been published in two peer-reviewed scientific manuscripts.
- We have examined PLAC1 transcriptional control in two ovarian cancer cell lines harboring different p53 mutations. PLAC1 transcription is a readout for WT p53 activity. We have further evaluated transcription, as shown below in Aim 3.
- We have performed Kinexus phosphoproteomic analysis of key cell cycle controllers in ovarian cancer cells (**Table 2**).

From these phosphorylation events (**Table 2, below**), we note the consistent enhancement of phosphorylation of two proteins, ABL and EZH2, when treated with anti-angiogenics bevacizumab and cediranib. The phosphorylation of ABL at Y257 inhibits the nuclear localization signal and prevents ABL from shuttling to the cytoplasm from the nucleus, which is required for normal cell proliferation. In addition, the phosphorylation of EZH2 on T487 is an inhibitory phosphorylation event that prevents EZH2 from stimulating cell proliferation. These are two novel signaling events associated with bevacizumab therapy that likely play a role in the improved outcomes noted when bevacizumab is combined with chemotherapy in GOG-218. On the other hand, a number of phosphorylation events are induced that appear to signal potential developing resistance effects, for example, the induction of insulin-like growth factor receptor (IGFR1) phosphorylation at 1161 and 1163 enhance IGFR1 kinase activity, potentially counteracting the therapeutic effects of bevacizumab and cediranib. These data suggest a novel therapeutic strategy to combine anti-angiogenics such as bevacizumab with tyrosine kinase inhibitors of IGFR1.

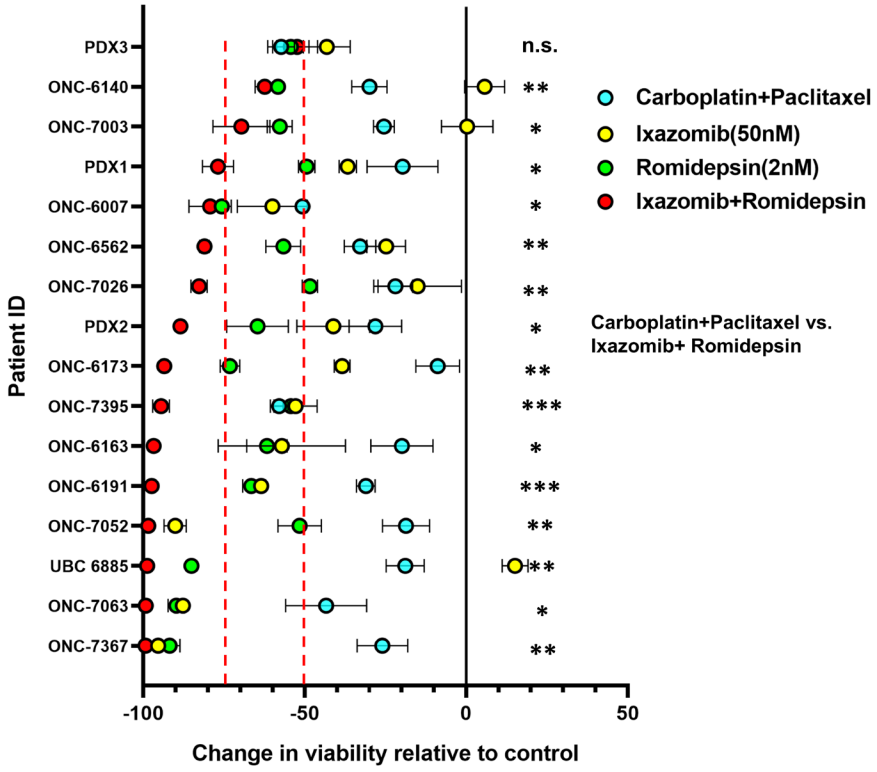
Table 2: Kinexus phosphoproteomic analysis of the impact of bevacizumab or cediranib on protein phosphorylation in CAOV3 ovarian cancer cells. Cells were treated with either bevacizumab or cediranib (1 μM), followed by assessment of protein phosphorylation using the Kinexus KAM phosphoproteomic array. The signal intensity was normalized to internal controls and calculated relative the untreated control. Lead candidates are identified. %CFC: percent change from control; data are colored from blue to yellow to indicate the significance of the change from control. Pan-specific indicates assessment of total protein expression.

		Control	Bevacizumab			Cediranib		
Target Name	P-Site	Average Normalized Net Signal	Average Normalized Net Signal	% CFC	Lead	Average Normalized Net Signal	%CFC	Lead
Abl (Abl1)	Y257	3761	7151	90	Priority	7167	91	Priority
Abl2 (Arg)	Pan-specific	6462	8429	30		10193	58	Possible
Abl2 (Arg)	Y439+T440	6574	13841	111	Possible	15058	129	Possible
ACC1 (ACACA)	S78+S80	475	778	64	Possible	717	51	
ACC2 (ACACB)	T1342	7026	14123	101	Possible	16933	141	Priority
ACTB	Y53	2575	3771	46	Possible	1777	-31	
AMPKa2 (PRKAA2)	Pan-specific	9034	7388	-18		13189	46	Possible
AMPKa2 (PRKAA2)	S377	4704	7193	53		9907	111	Priority
Arrestin b	S412	601	940	56	Possible	580	-4	
ATM	Pan-specific	9806	10319	5		15245	55	Possible
CDK9	Pan-specific	547	1015	85	Possible	424	-23	
CLK1	S337	3336	5296	59	Possible	3033	-9	
DLK (ZPK)	S269	8521	8510	0		2626	-69	Possible
ERK5 (MAPK7)	Pan-specific	5747	8469	47	Possible	9386	63	Possible
EZH2	T487	6183	11321	83	Priority	13512	119	Priority
FAK (PTK2)	Y397	3638	8418	131	Possible	5680	56	
FGFR3	Pan-specific	7669	7854	2		12534	63	Possible
FGFR4	Pan-specific	3838	4107	7		5629	47	Possible
Fgr	Pan-specific	11578	12896	11		17239	49	Possible
Flt3 (STK1)	Pan-specific	11462	10718	-6		17339	51	Possible
FOXK1	S441+S445	6302	10532	67	Possible	11558	83	
FOXO1A (FKHR)	S256	3907	4223	8		6029	54	Possible
GFAP	Pan-specific	1925	1849	-4		2829	47	Possible
HDAC5	S498	577	907	57	Possible	437	-24	
HePTP (PTPN7)	S44	5159	8929	73	Possible	4764	-8	
HGK (ZC1)	Pan-specific	1829	9151	400	Priority	7583	315	Priority
HGK (ZC1)	T187	1966	9240	370	Priority	6767	244	Priority
HIPK1	Y352	4323	7794	80		8040	86	Possible
HRAS	Pan-specific	3839	7555	97	Priority	6829	78	
HRAS	Y157	7782	9357	20		11801	52	Possible
IGF1R	Y1161+T1163	2644	5887	123	Priority	4878	84	Priority
ILK1 (ILK)	S343	2518	3820	52	Possible	2236	-11	
Jun (c-Jun)	S243	8758	9146	4		12759	46	Possible
Ksr1	Pan-specific	913	858	-6		381	-58	Possible
MAFG	S124	6485	23087	256	Priority	5204	-20	
MEK2 (MKK2, MAP2K2)	Pan-specific	6378	7041	10		9875	55	Possible
NFKB p65 (Rel A)	Pan-specific	1439	2569	79	Priority	1140	-21	
NuaK1 (ARK5)/Nuak2	T211	7180	8503	18		10713	49	Possible
p38a MAPK (MAPK14)	T180+Y182	9035	14318	58	Possible	11614	29	
p38d MAPK (MAPK13)	T180+Y182	437	1533	250	Priority	616	41	
p53 (TP53)	Pan-specific	4531	7503	66	Possible	5429	20	
p53 (TP53)	S37	1785	2588	45	Possible	2000	12	
p53 (TP53)	S392	6873	14237	107	Priority	5913	-14	
p70S6K (S6Ka, RPS6KB1)	Pan-specific	4504	8433	87	Priority	4950	10	
p70S6K (S6Ka, RPS6KB1)	T252	8986	17413	94	Possible	15747	75	Possible
PCYT1A (CTPCT; CCTA)	T342+S343	497	1571	216	Priority	721	45	
PDK1 (PDPK1)	S241	7643	16441	115		16849	120	Priority
PFKFB3	S461	1592	3034	91	Possible	1783	12	
Pim2	Pan-specific	610	535	-12		342	-44	Possible
PKCt (PRKCQ)	Y545	3949	7233	83	Priority	6334	60	Possible
PKG1a (PRKG1A)	T515+T517	5339	11329	112	Possible	5916	11	
PLCG1	Pan-specific	1087	669	-38		496	-54	Possible
Raf-B (BRaf)	Pan-specific	5927	8916	50		13809	133	Priority
ROCK2 (ROKa)	Y722	5566	3829	-31		2926	-47	Possible
SMG1	Pan-specific	6220	8675	39		10793	74	Possible
SSRP1	Y441+S444	1527	2629	72	Possible	1479	-3	
ULK2	Pan-specific	401	1013	153	Possible	37	-91	Likely due to Ab spot mis-print

Aim 2B: Identify the most effective molecular inhibitor to combine with chemotherapy in order to overcome the impact of each GOF mutant.

- We have performed a series of studies to interrogate various molecular agents in combination with chemotherapy based upon the specific *TP53* mutational status of the models. First, we found that the antiangiogenic agent cediranib is superior to bevacizumab (Bi et al., *Pharmaceuticals*).
- Having identified this alternative angiogenic, our findings set the stage for new clinical trials using cediranib or other tyrosine kinase inhibitor antiangiogenics in place of bevacizumab with chemotherapy.
- We found that p53 WT activity can be reactivated using a molecular reactivator that converts mutant p53 conformation back to wild type (Devor et al., *Pharmaceuticals*).

Figure 2: Sensitivity was calculated relative to untreated control.



- We also explored agents that induce massive cell death in ovarian cancer models and identified the combination of a histone deacetylase (HDAC) inhibitor with a proteasome inhibitor. We confirmed efficacy in cell lines, PDO models, and a xenograft model of a serous endometrial tumor, a histologic subtype that is very similar to high-grade serous ovarian cancer (example data are provided in **Figure 2** from Bi et al., *Cell Death and Disease)*.

Aim 2C: Determine the in vivo efficacy of the best combinations from Aim 2B.

- We have determined the in vivo efficacy of the combination of an HDAC inhibitor combined with a proteasome inhibitor in a xenograft model of serous endometrial cancer (**Figure 3**).

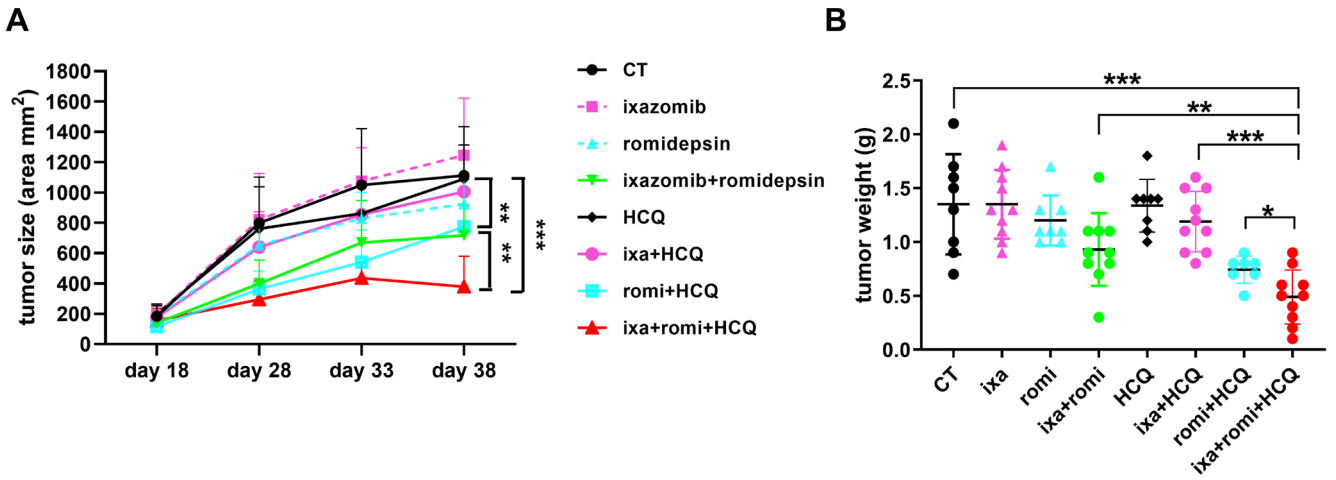
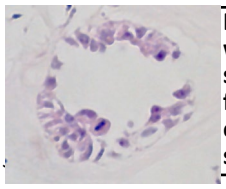


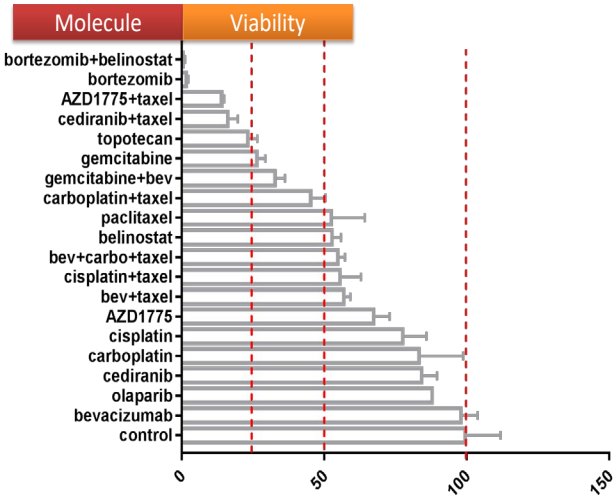
Figure 3: Sensitivity of xenograft models of Hec50 cells to ixazomib+romidespsin. A) Tumor volume; B) tumor weight at the completion of the experiment (Day 38).

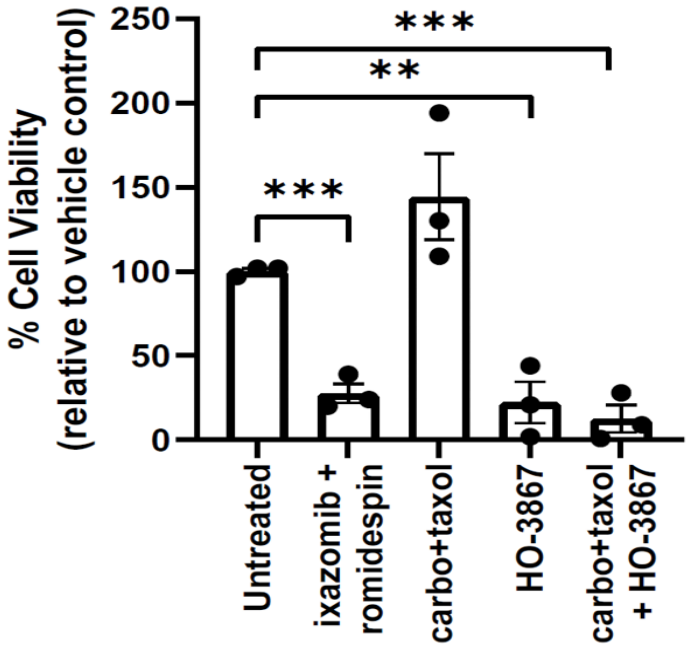
Aim 3: Interrogate the function of *TP53* mutations that are somatic variants of unknown function to define the best therapy for patients with these mutations.

- To date, we have binned 210 cases with *TP53* unique mutations in the GOG-218 dataset (**Figure 1**). Of these, nearly all of the 96 missense mutations are sVUFs. In addition, we have identified sVUFs in the PDO models of ovarian cancer that are also available for mechanistic studies.
- ***We have identified a highly active drug, HO-3867 (analogue of curcumin) and another agent, APR-246*** that demonstrate preclinical activity against sVUF and GOF p53 mutants (**Figures 4 and 5**).



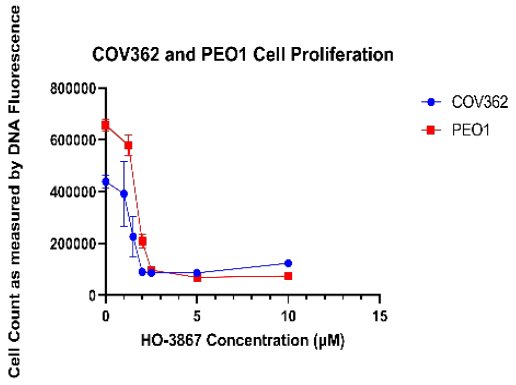
Example PDO. Tumor cells were cultured in matrigel supplemented with growth factors. After 5 days, cells were embedded in paraffin, sectioned and stained.





Drug concentration: Cisplatin 1µM, Carboplatin 1µM, paclitaxel 10nM, bevacizumab 1µM, AZD1775 125nM, Bortezomib 10nM, Belinostat 1µM, Olaparib 2µM, Gemcitabine 100nM, Topotecan 100nM, Cediranib 1µM.

Figure 4. Patient derived organoid expressing a mutation in *TP53* that is a somatic variant of unknown function (sVUF). Note the exquisite sensitivity to the curcumin derivative HO-3867 and its synergy with chemotherapy.



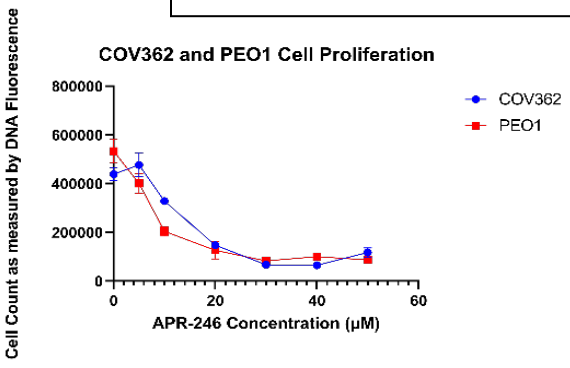


Figure 5. IC50 of HO-3867 and APR-246 in ovarian cancer cells with mutant p53 and BRCA. Concentrations that result in 50% cell death (IC50) are in the low µM range for both HO-3867 and APR-245.

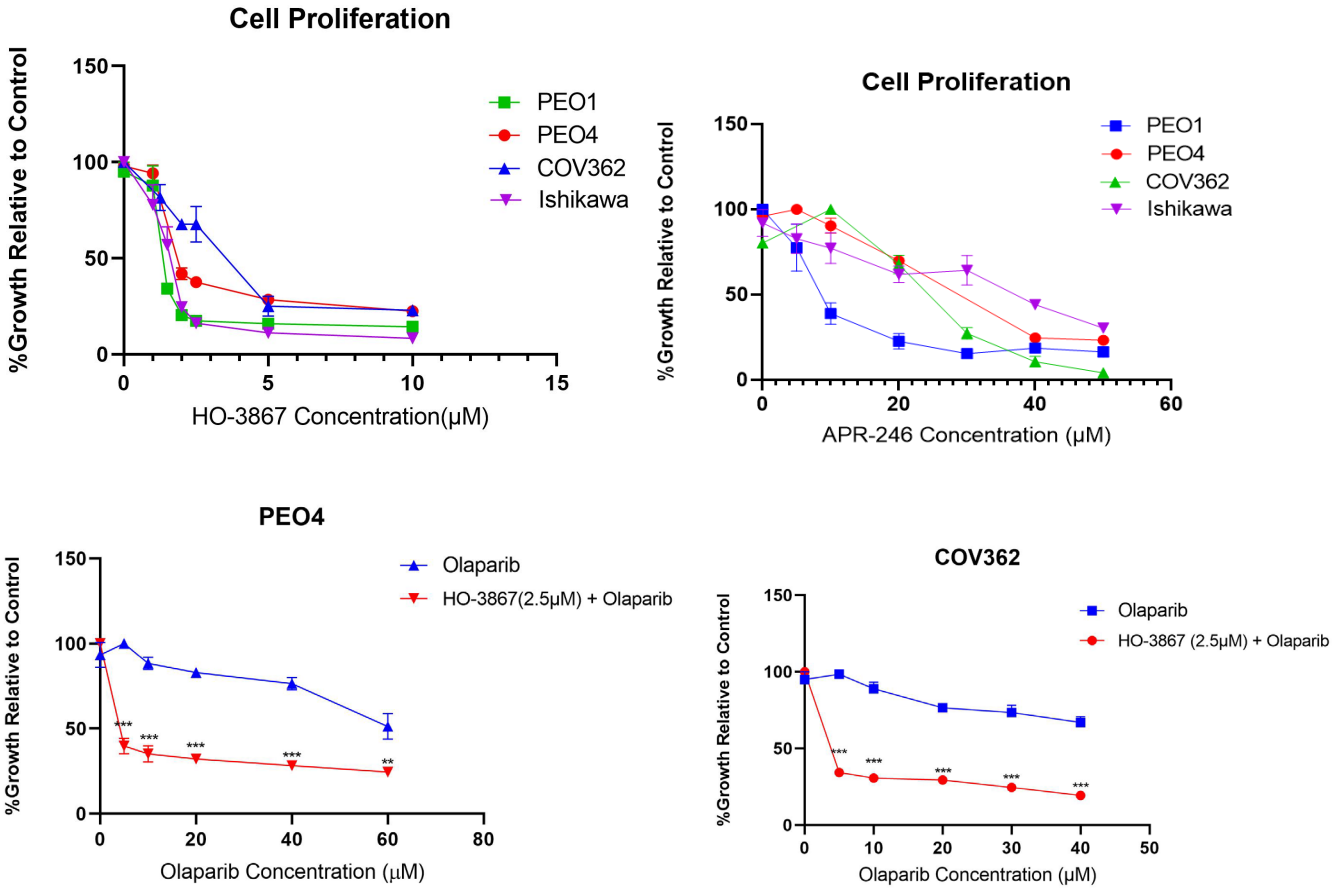
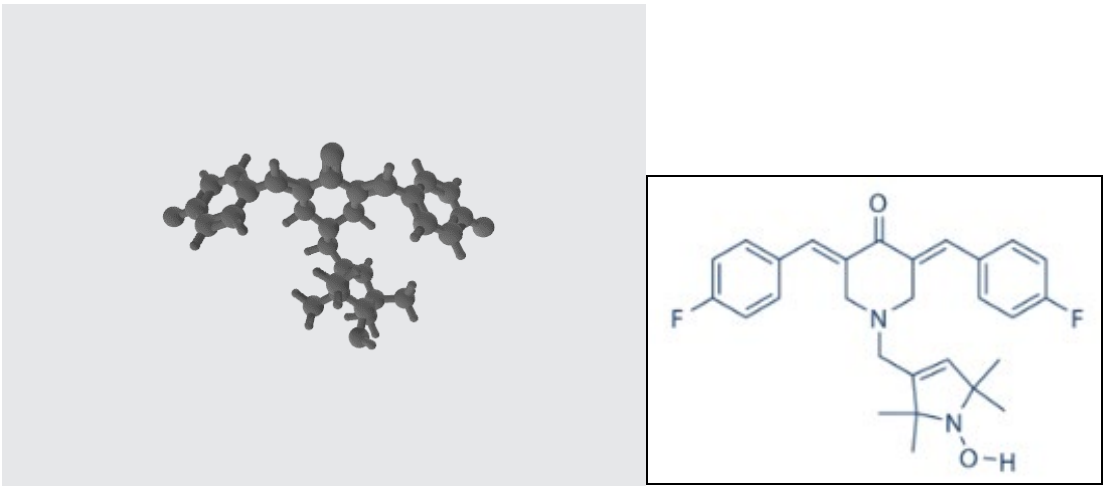


Figure 6. Top panel: IC50 experiments in multiple gynecologic cancer cell models demonstrating the IC50 of p53 reactivator therapies HO-3867 and APR-246. Bottom panel: the combination of HO-3867 and the PARP inhibitor, olaparib, create therapeutic synergy and significant enhance the effectiveness of olaparib in the cell models harboring mutations in p53.

The studies above demonstrate that in cells with sVUF and GOF mutations in TP53, the p53 reactivator HO-3867 has superior activity to another p53 reactivator, APR-246. In addition, HO-3867 synergizes with the PARP inhibitor olaparib to stave off resistance to PARP inhibitor therapy.

RNA sequencing has been performed to ascertain the molecular effects of p53 reactivator agents with significant clinical activity in cells with mutated *TP53.* In the presence of HO-3867, cells again induce the pro-apoptotic transcriptional program induced by normal, wild type p53, resulting in ovarian cancer cell death (without harming normal cells which continue to express normal forms of p53). We believe this work will underpin the development of new, less toxic therapies for ovarian cancer. We will continue to develop this important work over the coming year in the no cost extension period of this grant funding.



HO-3867 – the curcumin analogue now under study for anti-cancer properties in ovarian cancer models with mutant p53. Left = 3-D image; right = chemical structure

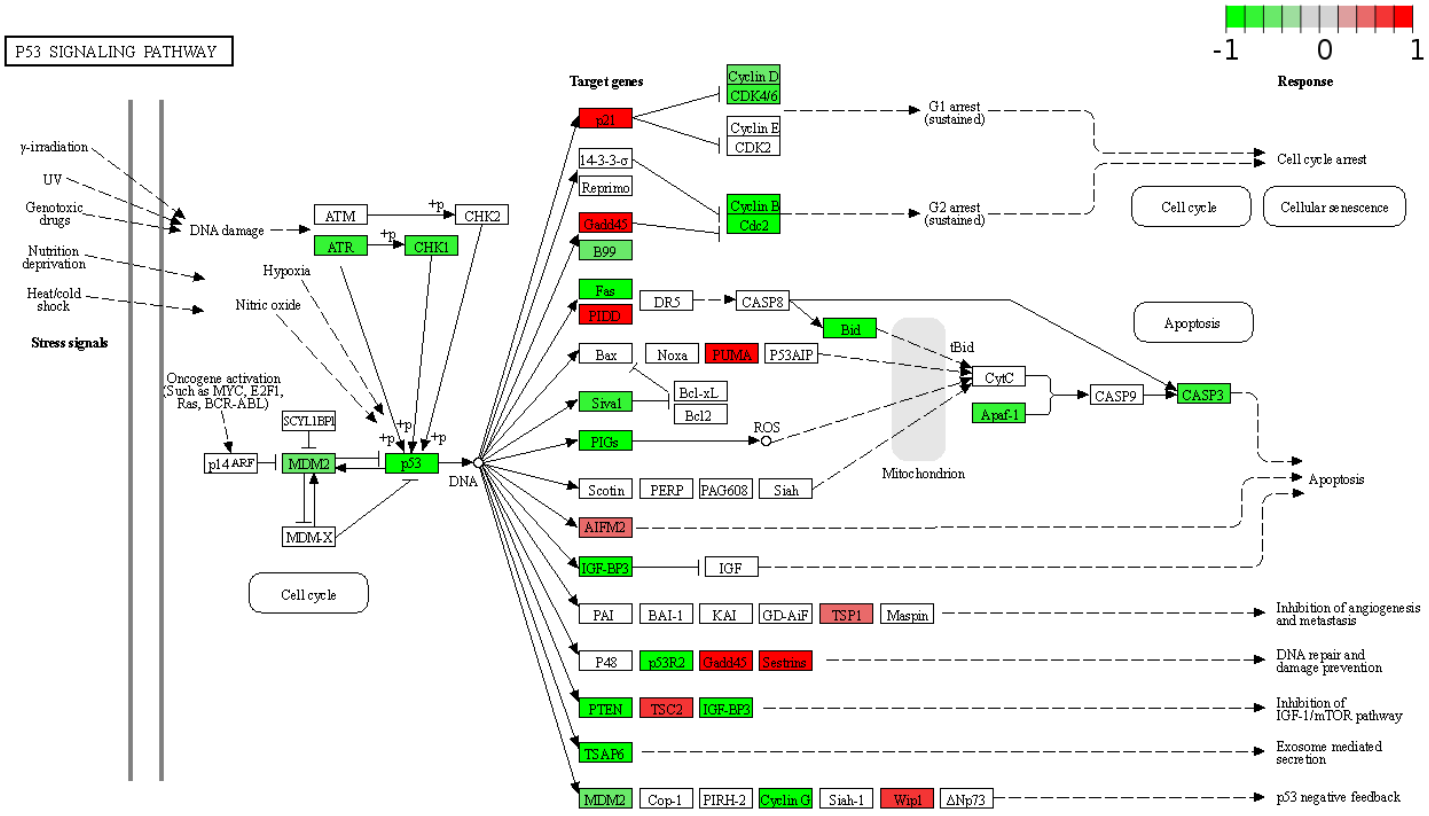


Figure 7. RNA-sequencing demonstrating re-instatement of normal, wild type p53 transcriptional effects in response to the p53 reactivating agent HO-3867 in ovarian cancer cell models. PE01 cells harboring a somatic missense mutation in *TP53*, G244D, and an inactivating mutation in BRCA 2 were treated with HO-3867 for 24 hours, mRNA was isolated and subjected to RNA-sequencing at the Genomics Shared Resource of the University of New Mexico Comprehensive Cancer Center. Significant alterations in gene transcription are noted that recapitulate the tumor suppressive, pro-apoptotic effects of p53 in response to HO-3867. HO-3867 is a curcumin analogue that binds to mutant forms of p53 and re-establishes the normal p53 conformation and transcriptional effects.

Major p53 pathway transcriptional effects of the mutant p53 reactivator HO-3867 include enforcement of the cell cycle checkpoints (upregulation of p21, Gadd45) and induction of cell death through apoptosis [upregulation of p53-induced

death domain (PIDD) and p53 upstream modulator of apoptosis (PUMA)]. The p53 self-regulatory negative feedback loop downregulating p53 expression was also re-established in response to HO-3867 treatment.
As a next step building upon the results obtained in Aim 3, we are pursuing the development of agents such as HO-3867 for clinical trials with an industrial partner. This will be an important and clinically meaningful future goal enabled by the current award.

What opportunities for training and professional development has the project provided?
This project was not intended to provide training and professional development opportunities. However, funds from this project have provided training opportunities for a postdoctoral fellow and a post-baccalaureate research intern. In addition, this project has supported in part the research of a clinical gynecologic oncology fellow in training. The purpose of this trainee's project was to create models of chemo-response using patient-derived organoids. A senior scientist on this grant (Co-investigator Dr. Kristina Thiel) has now attained an NCI K22 transition career development award and has accepted an independent faculty position at the University of Iowa. In addition, this grant supported the work of two summer undergraduate students and a high school student who both presented posters at the University of New Mexico Under-Represented Minority Poster Session, 2022 – 2023, winning top prizes for their work.

How were the results disseminated to communities of interest?
Results have been disseminated through 10 peer-reviewed publications. These results have also been presented in internal works-in-progress meetings at the sponsoring institution. Dr. Leslie was invited to review results from this study at the recent NIH meeting held in June, 2023, *Diverse Aspects of Uterine Serous Cancer*. This grant was acknowledged at that meeting. Results will also be presented at the AACR Ovarian Cancer meeting scheduled to be held in Boston this fall.

What do you plan to do during the next reporting period to accomplish the goals?
We will follow the experimental plan as outlined in the funded Project Narrative and the Statement of Work. Specific objectives in Aim 1 are to correlate p53 mutational status with outcomes through a collaboration with the NRG Oncology Statistical Office. For Aims 2 and 3, we will better characterize p53 mutations in PDO models harboring hotspot mutations from the GOG-218 cohort. We will also follow-up on the data from Aim 2 suggesting that the addition of IGFR1 inhibitors to bevacizumab may be a novel therapeutic strategy. We will also test inhibitors of EZH2 and ABL as new therapies in the models. Finally, having identified a potentially active agent in ovarian cancer, HO-3867, we will continue to pursue preclinical studies with this drug in preparation for future clinical trials.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?
These studies are anticipated to have a significant positive impact on the field by enhancing the design and choice of therapy for ovarian cancer based on specific *TP53* mutations. Moreover, our findings have the potential for immediate clinical application since we are performing retrospective analyses of a positive trial to better define a subgroup of patients that are more likely to respond. We will also prioritize drug choice in Aims 2 and 3 to use agents that have proven safety profiles and efficacy in other cancer types. Hence, our findings can be quickly implemented to provide personalized precision treatment options and improve outcomes for women with ovarian cancer using currently defined and FDA-approved treatments.

What was the impact on other disciplines? These results pertain to other cancers because many other cancer types have identical mutations in *TP53.* For example, our group has published on p53 mutations as predictors of response to bevacizumab-containing chemotherapy in advanced or recurrent endometrial cancer (Leslie et al., Gynecol Oncol 2021 and Thiel, et al., J of Clin Oncol, 2022).

What was the impact on technology transfer? Future collaborations with pharmaceutical companies is envisioned to develop alternative therapies for p53-mutated ovarian cancer.

What was the impact on society beyond science and technology? This grant supported the studies of under-represented minority students who are now going on to graduate and medical school. The future impact is expected to contribute to the development of the next generation of physician scientists who are from rural and Hispanic backgrounds.

5. Changes/Problems

Dr. Leslie has made a successful transition from the University of Iowa to the University of New Mexico Health Sciences Center. Her laboratory is now well established and continuing these studies.

6. Products

Publications, conference papers, and presentations

Journal publications:

1. Bi J, Newtson AM, Zhang Y, Devor EJ, Samuelson MI, Thiel KW, Leslie KK. Successful Patient-Derived Organoid Culture of Gynecologic Cancers for Disease Modeling and Drug Sensitivity Testing. Cancers (Basel). Published on 2021 Jun 10;13(12):2901. doi: 10.3390/cancers13122901. PMID: 34200645; PMCID: PMC8229222. Acknowledgement of federal support: yes
2. Bi J, Dixit G, Zhang Y, Devor EJ, Losh HA, Newtson AM, Coleman KL, Santillan DA, Maretzky T, Thiel KW, Leslie KK. Advantages of Tyrosine Kinase Anti-Angiogenic Cediranib over Bevacizumab: Cell Cycle Abrogation and Synergy with Chemotherapy. Pharmaceuticals (Basel). Published on 2021 Jul 16;14(7):682. doi: 10.3390/ph14070682. PMID: 34358108; PMCID: PMC8308742. Acknowledgement of federal support: yes.
3. Devor EJ, Schickling BM, Lapierre JR, Bender DP, Gonzalez-Bosquet J, Leslie KK. The Synthetic Curcumin Analog HO-3867 Rescues Suppression of PLAC1 Expression in Ovarian Cancer Cells. Pharmaceuticals (Basel). Published on 2021 Sep 21;14(9):942. doi: 10.3390/ph14090942. PMID: 34577642; PMCID: PMC8465575. Acknowledgement of federal support: yes.
4. Bi J, Zhang Y, Malmrose, PK, Losh HA, Newtson AM, Devor EJ, Thiel KW, Leslie KK. Blocking autophagy overcomes resistance to dual histone deacetylase and proteasome inhibition in gynecologic cancer. Cell Death and Disease (2022) 13:59. Acknowledgement of federal support: yes.
5. Ghezelayagh TS, Penington KP, Norquist BM, Khasnavis N, Radke MR, Kilgore MR, Garcia RL, Lee M, Katz, R, Leslie KK, Risques RA, Swisher EM. Characterizing TP53 mmutations in ovarian carcinomas with and without concurrent BRCA1 or BRCA2 mutations. Gynecol Oncol 2021, 160(3): 786-792. Acknowledgement of federal support: yes.
6. Gonzalez Bosquet J, Devor EJ, Newtson AM, Smith BJ, Bender DP, Goodheart MJ, McDonald ME, Braun TA, Thiel KW, and Leslie KK. Creation and validation of models to predict response to primary treatment in serous ovarian cancer. Scientific Reports (2021) 11:5957. Acknowledgement of federal funding: yes.
7. Thiel KW, Devor EJ, Filiaci VL, Mutch D, Moxley K, Alvarez Secord A, Tewari KS, McDonald ME, Mathews C, Cosgrove C, Dewdney S, Aghajanian C, Samuelson MI, Lankes HA, Soslow RA, and Leslie KK. TP53 sequencing and p53 immunohistochemistry predict outcomes when bevacizumab is added to frontline chemotherapy in endometrial cancer: An NRG Oncology/Gynecologic Oncology Group Study. J Clin Oncol 40:3289-3300, 2022. Acknowledgement of federal funding: yes.
8. **Leslie KK**, Thiel K. Reply to E. Guerra et al. J Clin Oncol. 2023 Jan 1;41(1):147. doi: 10.1200/JCO.22.01656. Epub 2022 Sep 16.PMID: 3611296. Acknowledgement of federal funding: yes.
9. Bi J, Zhang Y, Malmrose PK, Losh HA, Newtson AM, Devor EJ, Thiel KW, **Leslie KK.** Blocking autophagy overcomes resistance to dual histone deacetylase and proteasome inhibition in gynecologic cancer. Cell Death Dis. 2022 Jan 17;13(1):59. doi: 10.1038/s41419-022-04508-2. PMID: 35039480. Acknowledgement of federal funding: yes.
10. Gonzalez-Bosquet J, Cardillo ND, Reyes HD, Smith BJ, **Leslie KK**, Bender DP, Goodheart MJ, Devor EJ. Using Genomic Variation to Distinguish Ovarian High-Grade Serous Carcinoma from Benign Fallopian Tubes. Int J Mol Sci. 2022 Nov 26;23(23):14814. doi: 10.3390/ijms232314814.PMID: 36499142. Acknowledgement of federal funding: yes.

Poster Presentations

Star Tahy, Jamie Padilla, Lanie Smith and Kimberly K. Leslie. A novel mechanism of action of anti-angiogenic therapy: inhibition of the epigenetic modulator EZH2. Presented at the Student Poster Session, the University of New Mexico, 2022. Acknowledgement of federal support: yes.

Angelina Licor, Lanie Smith, Jamie Padilla, Vernon S. Pankratz, Kimberly K. Leslie. Efficacy of olaparib and p53 reactivators, separately and combined, to overcome PAPR inhibitor resistance in ovarian cancer cells. Presented at the Student Poster Session, the University of New Mexico, 2022. Acknowledgement of federal support: yes. This poster won a "Best Poster" award.

Alexander Goss, Jamie Padilla, Lanie Smith and Kimberly K. Leslie. Novel curcumin analogues as therapies for gynecologic cancer with *TP53* mutations. Presented at the Student Poster Session, the University of New Mexico, 2023. Acknowledgement of federal grant support: yes.

Other Products

Research material: Patient-derived organoid models of ovarian cancer of differing histologies (high-grade serous, carincosarcoma, clear cell, borderline).

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name	Role	Person months	Institution
Kimberly K. Leslie, MD	PI	No change	University of Iowa
Kristina W. Thiel, PhD	Co-I	No change	University of Iowa
Barbara Norquist, MD	Co-I	No change	University of Washington
Eric Devor, PhD	Co-I	No change	University of Iowa

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. Leslie received a new endometrial cancer grant from the CDMRP (CA210610) which does not overlap with this ovarian cancer project and a CDMRP Translational Team Science Award (CA220729), Progestin therapy for Endometrial Cancer, with no overlap with the current ovarian cancer award.

What other organizations were involved as partners? Nothing to report other than the known collaboration with the University of Washington, as approved with the initial submission.

8. Special Reporting Requirements

Not applicable.