AWARD NUMBER: W81XWH-21-1-0331

TITLE: Development of UR238, the First Small-Molecule Inhibitor of HE4 for Treatment of Ovarian Cancer

PRINCIPAL INVESTIGATOR: Richard G. Moore, MD

CONTRACTING ORGANIZATION: University of Rochester, Rochester, NY

REPORT DATE: August 2023

TYPE OF REPORT: Final

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

REPORT DOCUMENTATION PAGE		*Form Approved OMB No. 0704-0188*

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

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
August 2023	Final	01May2021-30Apr2023

4. TITLE AND SUBTITLE	**5a. CONTRACT NUMBER**
Development of UR238, the First Small-Molecule Inhibitor of HE4 for Treatment of Ovarian Cancer	W81XWH-21-1-0331
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)	**5d. PROJECT NUMBER**
Richard G. Moore, MD	**5e. TASK NUMBER**
E-Mail: Richard_Moore@urmc.rochester.edu	**5f. WORK UNIT NUMBER**

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	**8. PERFORMING ORGANIZATION REPORT**
University of Rochester 601 Elmwood Ave, Rochester, NY, 14642, USA	

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)	**10. SPONSOR/MONITOR'S ACRONYM(S)**
U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012	**11. SPONSOR/MONITOR'S NUMBER(S)**

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Epithelial Ovarian Cancer (EOC) is the most lethal gynecologic malignancy that women face. Novel targeted therapies for the treatment of EOC are urgently needed. This study aimed to develop the first-in-field novel therapeutic, UR238, designed to target the HE4 protein that is overexpressed in EOC and other HE4 overexpressing cancers. The Rationale for this project stemmed from our ongoing and published research that shows HE4 overexpression in tumors leads chemo-resistance and decreased survival for patients with EOC. Our research also shows that HE4 overexpression promotes expression of the immune checkpoint inhibitor ligand PD-L1 on tumor cells and on tumor associated macrophages, reduces CD8+T cell infiltration into the tumor microenvironment and induces an immune suppressive myeloid switch enriching the environment with immune suppressive M2 macrophages. Each one of these effects assists the tumor in evading the host immune system. The development of UR238 targeting HE4 will mitigate HE4 orchestrated immune evasion in EOC and will introduce a new class of therapeutics to treat EOC. Approximately 80% of patients with EOC tumors overexpress HE4 and these patients may receive clinical benefits from UR238. In addition, patients with endometrioid and serous endometrial cancers along with pancreatic and lung cancer patients also have tumors that overexpress HE4 and will likely benefit from UR238 targeted therapy.

15. SUBJECT TERMS

None listed.

<table>
<tr><td colspan="3">16. SECURITY CLASSIFICATION OF:</td><td rowspan="2">17. LIMITATION OF ABSTRACT

Unclassified</td><td rowspan="2">18. NUMBER OF PAGES

16</td><td>19a. NAME OF RESPONSIBLE PERSON
USAMRDC</td></tr>
<tr><td>a. REPORT

Unclassified</td><td>b. ABSTRACT

Unclassified</td><td>c. THIS PAGE

Unclassified</td><td>19b. TELEPHONE NUMBER (include area code)</td></tr>
</table>

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

TABLE OF CONTENTS

1. **INTRODUCTION:** This study aimed to develop the first-in-field novel therapeutic, UR238, designed to target the HE4 protein that is overexpressed in Epithelial Ovarian Cancer (EOC). The Rationale for this project stemmed from our ongoing and published research which shows HE4 overexpression in tumors leads to chemo-resistance and decrease survival for patients with EOC. Our research also shows that HE4 overexpression promotes expression of the immune checkpoint inhibitor ligand PD-L1 on tumor cells and on tumor associated macrophages, reduces CD8+T cell infiltration into the tumor microenvironment and induces an immune suppressive myeloid switch to inactive M2 macrophages. Each one of these effects essentially make the tumor invisible to being attacked by the host immune system. Novel targeted therapies directed to the treatment of EOC are urgently needed. A targeted therapy against HE4 will mitigate HE4 orchestrated immune evasion in EOC and lead to introduction of a new class of therapeutics to treat EOC. In terms of the *basic science scope*, UR238 serves as the proof-of-concept that HE4 is a targetable protein. In terms of the *clinical scope*, approximately 80% of patients with EOC tumors overexpress HE4 and will receive clinical benefits from UR238 targeted therapy. Further, patients with endometrioid and serous endometrial cancers along with a subset of pancreatic, lung and breast cancer patients also have tumors that overexpress HE4 and will likely benefit from UR238 targeted therapy.

2. **KEYWORDS:** HE4 targeted therapy, HE4, biomarker, PD-L1, immune checkpoint, CD8+T cells, macrophages, small molecule inhibitor, chemoresistance, survival, M2.
3. **ACCOMPLISHMENTS:**

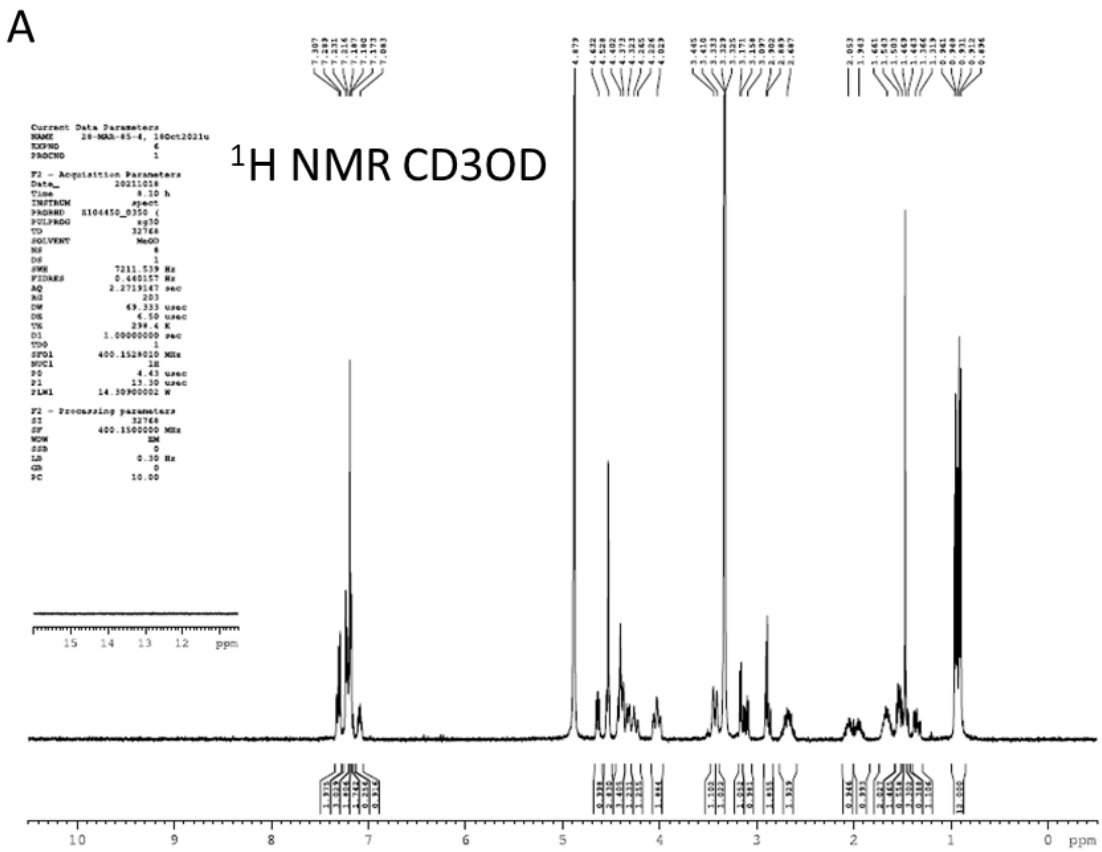
Major Goals:

Specific Aim-1:

Specific Aim 1:	**major objectives**	**Timeline Months**	**Completion**	**Completion %**
Subtask-1	To synthesize 2 gm of UR238	05/2021-11/2021	12/20/2021	100%
Subtask-2	Run HE4 ELISA assay to screen and select HE4 overexpressing ovarian cancer cell-lines	12/2021- 01/2022	01/2022	100%
Subtask-3	To determine the effect of UR238 treatment on HE4 secretion in HE4 overexpressing cell-lines in vitro and in vivo alone or in combination with paclitaxel 1. vehicle (n=10, IP, M-F) 2. UR238 (n=10, 5mg/kg, IP, MWF) **3.** Paclitaxel (n=10, 5mg/kg, IP, Monday) **4.** UR238 (n=10, 5mg/kg, IP, MWF)+paclitaxel (n=10, 5mg/kg, IP, M)	12/2021- 02/2022	Completed on time	100%

Significant results:

SubTask-1.1: Development of industrial synthesis of pharmacologic grade UR238 in 2-grams quantities was achieved. The free base was first synthesized in 94% purity. To reduce the impurities, improve the purity, and additionally impart greater solubility, HCl salt of UR238 in 94.59% HPLC purity was synthesized. A total of 2 grams was synthesized opposed to the manufacture of proposed 500mg. The ^{1}HNMR and HPLC profiles of UR238-HCL salt are shown below (Figure-1A and Figure-1B).



	RT	Area	% Area	Height
6	8.958	3219305	94.59	434199
7	9.291	8726	0.26	2088
8	9.411	18015	0.53	6147
9	9.456	4333	0.13	2682
10	9.508	7003	0.21	2853
11	9.556	3171	0.09	1174
12	11.919	23951	0.70	7904
13	13.588	7113	0.21	1686

Figure-1: (**A**): ^{1}HNMR profile of UR238-HCL salt. (**B**): HPLC profile of UR238-HCL salt.

Methodology: The contractor (TRC Canada, Inc.) has not yet disclosed the schema and the methodology of synthesis in detail despite requests made.

Explanations for significant delays: The contractor (TRC Canada Inc.) experienced significant difficulties in purification of UR238 free base which delayed the delivery. The highest purity achieved of UR238 free base was 94.59%.
Resolution of purity challenges: Facing the persistent difficulties with achieving >95% purity of free base UR238, we proposed to the contractor the synthesis of HCL salt as the way to improve the purity and also to improve the aqueous solubility. The contractor synthesized HCL salt in 94.59% purity (Fig-1B) and shipped to us on 12/16/2023. This material was used in all the studies presented henceforth.

<u>SubTask-1.2</u>: Identification of HE4 overexpressing cell-lines and determination of the responses of UR238 on HE4 secretion and expression in ovarian cancer cell-line panels in vitro: OVCAR-3, CaOV-3 and HCH-1 were identified as the natural high-HE4 expressor and secretory cell-lines**.** UR238 (0.5 and 1µM) treatment reduced HE4 expression in NUTU19 and SKOV-3 cells (Figure-2A)**.** UR238 (1µM) decreased nuclear expression of HE4 in SKOV-3 cells (Figure-2B). UR238 decreased HE4 secretion levels in SKOV-3-SH1 high expressor clones (Figure-2C). UR238 treatment decreased HE4 expression in OVCAR-3 cells at 0.1µM) without affecting the viability (Figure-2D). Similarly, UR238 treatment at 31.25, 62.5 and 125nM reduced the secretion of HE4 in HCH1 ovarian cancer cells (Figure-2E).
Brief description of Methodologies:
Detection of HE4 expression in ovarian cancer cell-lines: Using western blot and an ELISA kit, we screened the panel of ovarian cancer cell-lines to select OVCAR-3, CAOV-3 and HCH-1 as the natural high-HE4 expressors. In addition, we stably transfected SKOV-3, a low expressor cell-line, to generate SKOV-3SH1, an HE4 overexpressing cell-lines.
Challenges: None

<u>Subtask-1.3</u>: To determine the effect of UR238 treatment on HE4 secretion in HE4 overexpressing cell-lines in vitro and in vivo alone or in combination with paclitaxel.
Significant results: UR238 treatment reduced HE4 total expression in human SKOV-3 and rat NUTU-19 ovarian cancer cells (Figure-2A), nuclear expression in SKOV-3 (Figure-2B), reduced HE4 production in SKOV-3SH1 cells (Figure-2C) and reduced secretion of HE4 in OVCAR-3 and HCH-1 ovarian cancer cells (Figure-2D and -2E).
However, co-treatment of UR238 with paclitaxel did not generate a synergistic response in SKOV-3 xenograft model (Figure-3).

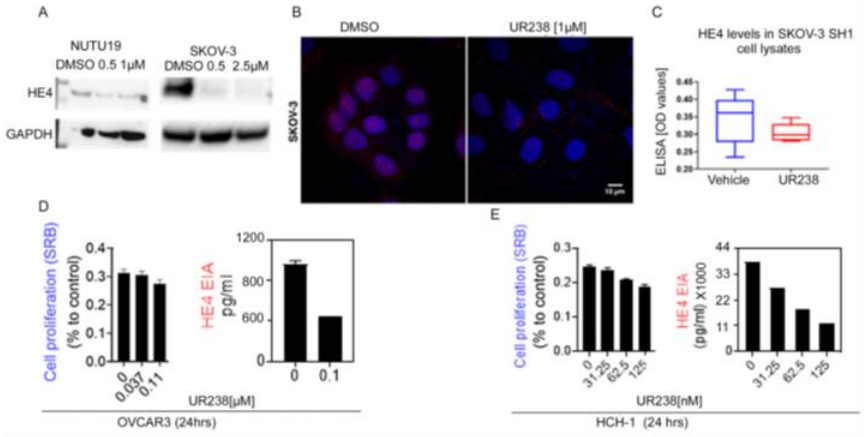


Figure-2: (A): UR238 treatment reduced expression of HE4 in NUTU19 and SKOV-3 cells. (**B**): confocal microscopy showed that UR238 treatment reduced nuclear HE4 expression in SKOV-3 cells. (**C**): UR238 treatment reduced HE4 expression levels in the lysates of SKOV-3-HE4 overexpressed clone SKOV-3SH1 within 6 hours of treatment without affecting the cell-viability. (**D**): UR238 reduces HE4 production in HCH-1 cell lysates at 100nM without majorly affecting viability of OVCAR-3. (**E**): UR238 reduces HE4 production in HCH-1 cell lysates at doses 31-125nM during 24 hr. treatment (Stains: DAPI-blue and HE4-red).

Methods for detection of HE4 expression in ovarian cancer cell-lines after UR238 treatment: The expression of HE4 in NUTU19 and SKOV-3 EOC post UR238 treatment was examined by western blot analysis of the lysates of the cells treated with vehicle (DMSO) or UR238. The PVDF membranes were proved with HE4 antibody or GAPDH to establish the loading homogeneity.

Methods for Confocal microscopy: The effect of UR238 on the nuclear or cellular expression was detected by confocal microscopy. Vehicle/UR238 treated SKOV-3 cells were fixed, permeabilized and stained with HE4 primary antibody (24 hours), washed, and stained with secondary-linked with fluorophore. The washed cells were stained with DAPI containing mounting medium and examined with a confocal microscope.

Methods for Figure-2C: SKOV3SH1 HE4 overexpressing cells were treated with vehicle/UR238 for 6-hours. The cells were lysed using a commercially available lysis buffer. The clarified lysates were analyzed using a US FDA approved ELISA kit (Fujirebio Inc.) (B): UR238 combined with paclitaxel did not exhibit increased inhibition of tumor growth in SKOV-3 xenograft model.

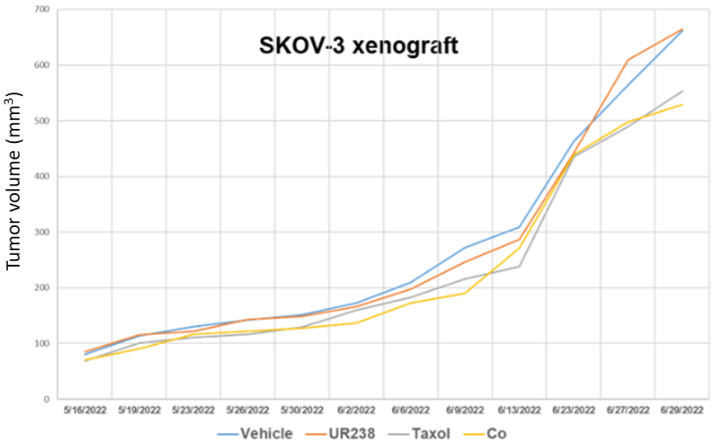


Figure-3: Evaluations of synergistic activities of UR238 with paclitaxel. UR238 combined with paclitaxel did not exhibit increased inhibition of tumor growth in SKOV-3 xenograft model.

Methods for Figure-2D: Cell population was assessed by the sulforhodamine-B (SRB) assay. In brief, OVCAR-3 and HCH-1 HE4-positive cell lines were exposed to UR238 at the indicated concentrations. After 24 hours, cells were fixed with cold 5% trichloroacetic acid (TCA) at 4°C for 1 hour. The fixed cells were then stained with a 0.057% SRB solution for 30 minutes at room temperature, followed by four washes with 1% acetic acid. Subsequently, the protein-bound SRB dye was solubilized using a 10 mM Tris base solution. The optical density of each well was measured at 510 nm.

Methods for Figure-2E: For HE4 levels, OVCAR-3 and HCH-1 cells treated with UR238 were lysed, and protein extracts were prepared and quantified using the Bio-Rad DC protein assay. The HE4 levels in an equal amount of proteins were determined using a human HE4 ELISA kit (R&D Systems) following the manufacturer's recommendations.

Methods for Figure-3: SKOV-3 (1-million/animal) were implanted subcutaneously in NSG mice. After 1-week when tumors had become palpable, tumor sizes were measured and mice were treated with vehicle, UR238, paclitaxel or treatment in combination with paclitaxel+UR238 (M-F, IP). Tumor sizes were measured periodically.

Milestones: All milestones achieved

Challenges: none

Delays: Manufacture of UR238 was delayed by 20 days.

Specific Aim-2:

Tasks	major objectives	Timeline Months	Completion month	Completion %
Subtask-1	Treat the animal groups per following schedule: 1. HE4+ group +vehicle (n=10, IP, daily) 2. HE4+ group +UR238 (n=10, IP, daily)	01/2022-8/2022	8/2022	100%
Subtask-2	Harvesting of the blood, tumors, and spleen. Analysis of the venous blood, tumor and spleen cells using Flow cytometry of tumors and immune cells using mice antibody panel. Luminex analysis of serum	03/2022	03/2022	100%
Subtask-3	Data interpretation and presentation in reports and publications.	03/2022	Completed on time	100% Manuscript in preparation

Significant results-2: Under the aegis of Subtask-2, we evaluated the response of UR238 using syngeneic rat ovarian cancer model. Syngeneic rat EOC model closely recapitulates key pathologic features of EOC. As shown in Figure-4: A-B, UR238 treatment reduced ascites production, and omental tumor deposition. Images of rats with EOC tumor nodules in the vehicle groups compared to the absence of tumor nodules in the omentum of rats treated with UR238 are shown in Figure-4:C-D. UR238 reduced the population of immune suppressive granulocytes in the omentum and ascites (Figure-4: E, G), and PD-L1 expression on tumor cells both in the omentum and ascites (Figure-4: F&H).

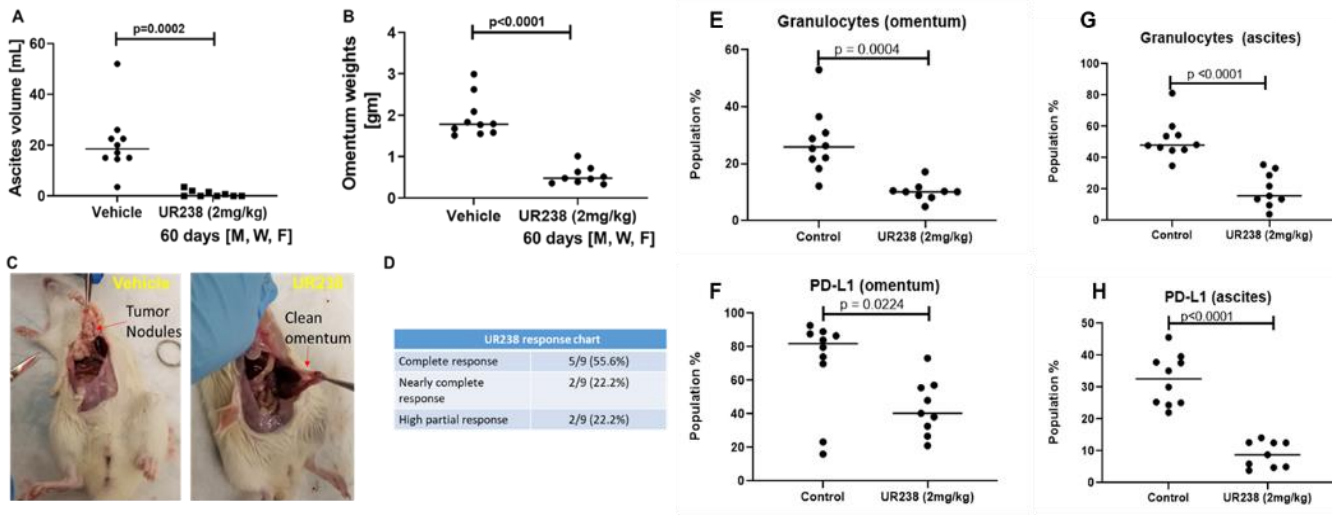


Figure-4: HE4 overexpressing NUTU19 clone cell were inoculated in Fisher344 rats. Rats were treated with vehicle or UR238 (2mg/kg, MWF, IP). Analysis of the euthanized rats show that: (**A**)- UR238 treatment reduced ascites volume in rats significantly. (**B**): UR238 treatment reduced tumor burden on omentum significantly. (**C**): An image of tumor ridden (vehicle, left) and tumor free (UR238 treated) omentum is shown. (**D**): Complete response in 5/9 rats, nearly complete response in 2/5 and major response in remaining 2/9 rats was observed upon UR238 treatment. (**E**): UR238 treatment reduced granulocytes in the omentum of the tumor inoculated rats. (**F**): UR238 treatment reduced PD-L1 on the tumor cells in the omentum of the tumor inoculated rats. (**G**): UR238 treatment reduced granulocytes in the ascites of the tumor inoculated rats. (**H**): UR238 treatment reduced PD-L1 expressed on the tumor cells in the ascites.

Methods for Figure-4: To determine the effects of UR238 treatment on the tumor immune microenvironment and tumor and ascites burden of EOC tumors in immune competent rats, HE4 overexpressing Nutu-19 clones were implanted in Fisher344 rats and treated with UR238 (2mg/kg) or vehicle. Animal weights were measured twice a week. On day 60 from inoculation, the rats were sacrificed, and tumors, ascites and peripheral blood were harvested. Tumors were imaged and weighed on a calibrated balance. Tumors were disintegrated into single cell suspension. Single tumors cells and immune cells from tumors, ascites and peripheral blood were analyzed by flow cytometry and analyzed by FlowJo software.
Milestones: All the milestones achieved timely
Challenges: none
Delays: none

Specific Aim-3:

Tasks	**major objectives:** **To determine the effect of UR238 on tumor growth and immune structure human PDX model in NSG mice**	**Timeline Months**	**Completion month**	**Completion %**
Subtask-1	Recruitment of 5 high grade serous EOC patients with high HE4 expression	7/2021-9/2022	12/2022	100%
Subtask-2	• Inoculate mice with minimally processed tumor tissues. • Treat the animal groups per following schedule for humanized xenografts, each: 1. Vehicle (n=10, IP, MWF) 2. UR238 (n=10, 5mg/kg, IP, MWF)	12/2022-01/2023	Implanted 3 different cohorts of mice timely. Model still under development	80%
Subtask-3	Harvesting of the blood, tumors, and spleen. Analysis of the venous blood, tumor and spleen cells using Flow cytometry of tumors and immune cells using mice antibody panel.	12/2022-01/2023	Analysis of the lavage samples completed timely.	80%

Subtask-4	Data interpretation and presentation of reports.	12/2022-01/2023	incomplete	Manuscript in preparation

Methods: Fresh human tumor specimens and ascites cells from treatment naïve HE4 over expressing high grade serous EOC patients were either cultured or implanted in NSG mice. Mice were implanted with three independent patient EOCs at three different iterations with increasing tumor cell quantities.
Results: Even though we followed the published methodologies strictly, we did not observe any noticeable PDX tumor implantation on omentum and peritoneal wall in NSG mice despite three independent attempts. However, we were successful in detecting the presence of human tumor cells with lavage of the peritoneum. We were also able to treat a cohort of mice with UR238 as proposed. Figure-5 shows that NSG mice implanted with PDX cells did carry human EOC related immune cells (CD3/CD19 and CD14/CD11+) in peritoneal lavages. Further, presence of CD3/CD19+ and CD14/CD11+ cells were also detected in omentum. Moreover, UR238 treatment reduced the population of CD3CD19+ lymphocytes and CD14+/CD11+ cells (Figure-5) in the peritoneum lavages. Similarly, UR238 treatment reduced CD3/CD19+ve cells in omentum but CD14/CD11b cells population was increased by UR238 treatment.

To improve PDX implantation and circumvent the failure of xenografting of PDX tumors in NSG mice, near the end of the grant period we have developed a new process. Instead of injecting the tumor slurry containing larger size fat lumps which may have impeded the delivery of optimum number of tumor cells, we filtered and injected the slurry containing mostly tumor cells and immune cells. More recently in one mouse, in an ongoing experiment, we have detected tumor nodules in the omentum, indicating the preliminary success of our new PDX model. We are currently monitoring the progress and outcome of this approach and will be treating animals with UR238 once this model has been established.

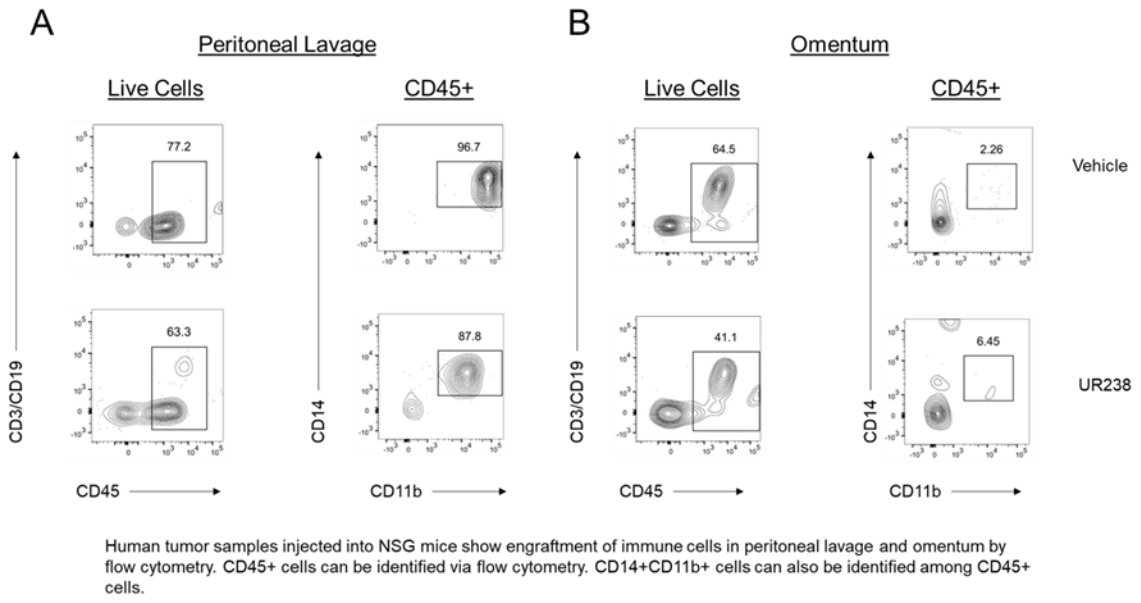


Figure-5: (A): peritoneal lavage shows presence of human immune cells (CD3/CD19 and CD14) and UR238 treatment reduced CD3/CD19 and CD14/CD11b populations. (B): Flow cytometry staining of mouse omentum showed the presence of human CD3CD19+ve and CD14/CD11b positive cells. While UR238 reduced CD3/CD19+ve population, CD14/CD11b population was found to be increased by UR238 treatment.

Moreover, this experiment provided us an opportunity to establish a novel ex-vivo model for PDX and test the activity of UR238 on HE4 and PD-L1, our key targets of interest. As shown below (Figure-6), UR238 inhibited the expressions of both HE4 and PD-L1 attesting our hypothesis that HE4 and PD-L1 are inter-linked in EOC signaling.

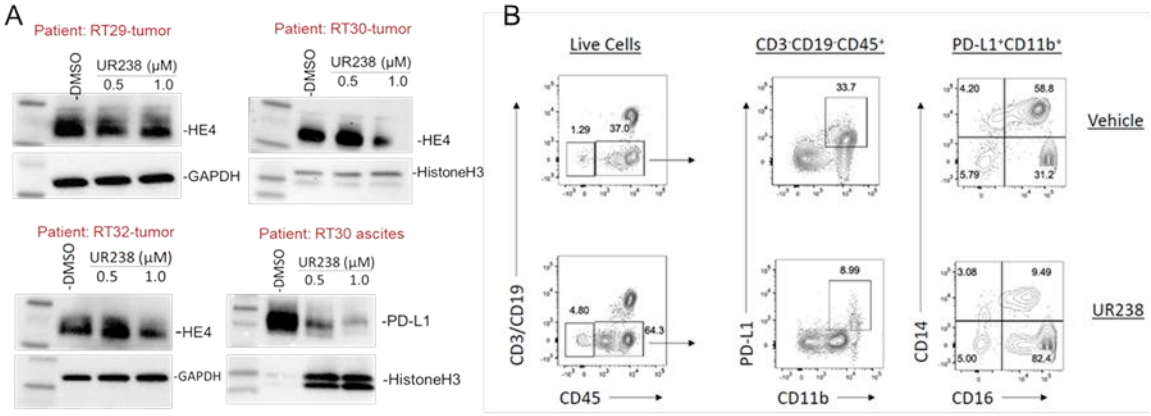


Figure-6: (A): UR238 treatment reduced PD-L1 expression in the tissue samples collected from a number of patients within 24 hours of the treatment at the indicated doses ex vivo. Both tumor cells and ascites cells showed decrease in PD-L1 expression upon UR2328 treatment *ex-vivo*. (B): Flow cytometry staining of sEOC patient-derived ascites cells cultured for 48 hrs with UR238 (1uM) or vehicle. Expression of PD-L1 among CD45+CD3-CD19- cells decreases with UR238 treatment. The population of CD14+CD16+ M2 macrophages decreases with UR238 treatment.

Milestones: All the milestones achieved timely.

Challenges: PDX tumors initially did not form successfully in NSG mice, despite 3 rounds of experimentations including extension of the monitoring period up to 200 days than 45-60 days initially proposed, per the published reports, which necessitated the optimization of the PDX process. Our currently optimized method has presented early indications of success in the PDX model.

Delays: none

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

This project provided training opportunities to:

1. Naixin Zhang MD: Dr. Zhang is a Gynecologic Oncology fellow and required by the ACGME fellowship to spend 1year of his 3-year fellowship performing basic science research. Dr. Zhang obtained training in all phases of this research.
2. Mr. John P. Miller: Mr. Miller is currently a PhD candidate through the University of Rochester. Mr. Miller thesis is based around the immune modulation of HE4 and its effects on the tumor microenvironment. Mr. Miller will use some of the data generated through this grant as part of his PhD thesis.

Describe how the results were disseminated to communities of interest.

Nothing to report: We anticipate publishing our results in a peer-reviewed scientific journal. A manuscript is in preparation.

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

Nothing to report. This is the final report.

4. IMPACT

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

The discovery that the WFDC2 gene and its protein product, HE4, is a viable druggable target for the treatment of overexpressing HE4 tumors introduces a new class of agents to treat cancers. This study is the first bench-to-bedside project with the development of a pharmaceutical grade HE4 inhibitor, UR238, that is positioned to enter pharmacokinetic and toxicity testing and subsequently into Phase 1 human clinical trials. UR238 was identified by an iterative HE4 reporter-based library screening with optimization through medicinal chemistry techniques to be used as a targeted therapy for the treatment of ovarian cancer and other HE4 overexpressing tumors. UR238 is the first-in-field, novel small molecule to validate HE4 as a druggable target for the treatment of HE4 over expressing tumors. This discovery may also lead to other approaches of targeting HE4 expressing tumors including other small molecules and monoclonal antibody targeted therapy which are currently being investigated in our laboratory. This novel class and approach to treating patients with epithelial ovarian cancer will also extend to other HE4 overexpressing tumors including endometrial, pancreatic, lung and some breast cancers.

What was the impact on other disciplines?

UR238 is a very potent and specific 20S-proteasome inhibitor. It is a 3-5-fold more potent 20S proteasome inhibitor than the clinically used carfilzomib. Therefore, in addition to ovarian cancer, endometrial and ductal breast cancer treatment, UR238 can also be used for the treatment of relapsed and or/refractory multiple myeloma.

What was the impact on technology transfer?

UR238 is being evaluated by at least two companies for licensing. At BIO-2023, conference in Boston MA, more than 7 companies showed interest in UR238. Notably, Empire Discovery Institute (EDI) has had multiple rounds of discussions and is continuing to evaluate the technology. Similarly, 7 Primus Partners Inc has held repeated rounds of discussions relating to licensing UR238.

What was the impact on society beyond science and technology?

This study provides the first-in-field proof of concept that HE4 is a targetable protein. This knowledge will allow biotechnology companies and other academic laboratories to search and develop new chemotypes of HE4 inhibitors. It is anticipated that targeting HE4 will improve survival of over 80% EOC patients who exhibit HE4 overexpression.

5. CHANGES/PROBLEMS

Actual or anticipated problems or delays and actions or plans to resolve them:

1. Achieving greater than 94% purity for UR238 posed a challenge for the contractor's laboratory. We resolved this problem by converting UR238 free base to HCL salt which generated additional benefit of improved aqueous solubility. This method led to the improvement of purity by 0.59% (total purity: 95.49%). In future, we plan to purify every intermediate in more than 98% purity before initiating next step of synthesis.
2. PDX model in NSG mice initially failed to set up. We attempted to resolve tumor uptake: 1): increasing the tumor slurry quantity; 2): increasing the number of mice, and 3): increasing the days to let tumors set up in mice. To improve PDX implantation and circumvent the failure of xenografting of PDX tumors in NSG mice, near the end of the grant period, we developed a new process. Instead of injecting the tumor slurry containing larger size fat lumps which may have impeded the delivery of optimum number of tumor cells, we filtered and injected the slurry containing mostly tumor cells and immune cells. More recently in one mouse, in an ongoing experiment, we have detected tumor nodules in the omentum, indicating the preliminary success of our new PDX model.

Changes that had a significant impact on expenditures.
Nothing to report.

Significant changes in the use or care of human subjects.
Nothing to report.

Significant changes in use of biohazards and/or select agents.
Nothing to report.

6. PRODUCTS:

Nothing to report. A manuscript is in development. Several abstracts have been submitted for presentation in the upcoming SGO Annual meeting on Women's Cancers.

Books or other non-periodical, one-time publications.
Nothing to report.

Other publications, conference papers and presentations.
Nothing to report.

Website(s) or other Internet site(s).
Nothing to report.

Technologies or techniques.

1. UR238, the novel small molecule inhibitor of HE4.
2. HE4 overexpressing cell-lines clones of SKOV-3.

Inventions, patent applications, and/or licenses.
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

1. Name: Richard Moore MD,
   Role: PI.
   ORCID: 0000-0001-5598-5263
   Person months worked: 1.8
   Contribution to the project: Overall supervision, resources, weekly meeting, experimental design, and data review and analysis.
   Funding support: none

2. Name: Kyu Kwang Kim PhD (Co-I)
   Role: Co-I
   ORCID: 0000-0002-0386-3712
   Person months worked: 1.74
   Contribution to the project: screening, immunoblotting, ELISA, cell viability, data presentation, review of data.
   Funding support: none

3. Rakesh Singh PhD, MBA (Co-I)
   Role: Co-I
   ORCID: 0000-0002-1124-9202
   Person months worked: 2.72
   Contribution to the project: synthesis of UR238, coordination with contract manufacturer of UR238, in vivo experiments, data presentation, review of data.

Funding support: none

4. Rachael Turner MD, PhD (faculty)
   Role: Co-I
   ORCID: 0000-0002-8726-4628
   Person months worked: 0.0
   Contribution to the project: in vivo rat EOC experiments, data presentation, review of data.
   Funding support: none

5. Naixin Zhang MD
   Role: Postdoctoral fellow
   ORCID: 0000-0001-8946-0318
   Person months worked: 0.0
   Contribution to the project: patient enrollment, procurement of tumor specimens, processing the tumors cells into a slurry, injections of slurry in NSG mice, treatment of mice with UR238, western blots.
   Funding support: none

6. Negar Khazan PhD (post-doctoral fellow)
   Role: Postdoctoral fellow
   ORCID: 0000-0002-5517-6017
   Person months worked: 4.1
   Contribution to the project: western blots and PCR.
   Funding support: none

7. Niloy Singh
   Role: Technician
   ORCID: NA
   Person months worked: 4.5
   Contribution to the project: Western blots, tumor harvest from rats, processing of the tumors.
   Funding support: none

8. Cameron Snyder (technician)
   Role: Technician
   ORCID:
   Person months worked: 0.0
   Contribution to the project: western blots, tumor harvest from rats, processing of the tumors.
   Funding support: none

8. John P Miller, graduate student
   Role: Graduate student
   ORCID: 000-0001-8786-5442
   Person months worked: 0.0
   Contribution to the project: tumor and ascites harvest.
   Funding support: none

9. Liz Lamere (technician)
   Role: Technician
   ORCID: NA
   Person months worked: 0.0
   Contribution to the project: Animal colony maintenance, tumor cell inoculations, treatment with UR238.
   Funding support: none

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative*

report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES

Nothing to report.