

**AWARD NUMBER:** W81XWH-20-1-0190

**TITLE:** Multimodal Treatment Consisting of Ferroptotic Agent and Chimeric TRAIL as a Second-Line Therapy for Ovarian Peritoneal Carcinomatosis

**PRINCIPAL INVESTIGATOR:** Yong J. Lee

**CONTRACTING ORGANIZATION:** University of Pittsburgh, Pittsburgh, PA

**REPORT DATE:** July 2022

**TYPE OF REPORT:** Annual

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Fort Detrick, Maryland 21702-5012

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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> Ovarian cancer is the most common cause of death among all gynecological neoplasms. Ovarian peritoneal carcinomatosis (OPC) is a frequent terminal evolution of ovarian cancer and is regarded as a lethal condition. In the past, OPC was considered a terminal disease stage, but over the past two decades, the therapeutic techniques of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemoperfusion (HIPEC) have been developed. However, although CRS treated with HIPEC has been associated with an increased response rate, complete responses have been rare and recurrences are common. In this grant application, we are developing a multimodal treatment consisting of the FDA-approved ferroptotic agent artesunate and TRAIL as a second-line therapy. Currently, we are establishing tumoroids of OPC and investigate the mechanism of the synergistic induction of apoptosis caused by the integration of signal transduction pathways in organoids of OPC. Although ferroptosis is considered a distinctive form of cell death compared to other types of death such as apoptosis and it is known to result from iron-dependent accumulation of lipid peroxides rather than caspase activation, our studies have shown that ferroptosis interplays with apoptosis. In this grant period.					
<b>15. SUBJECT TERMS</b> Ferroptosis, apoptosis, crosstalk between death signals, synergistic interaction					
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

In this grant application, we propose developing a humanized patient-derived xenograft (PDX) mouse model and tumoroids to assess the preclinical efficacy of chimeric TRAIL (tumor necrosis factor-related apoptosis-inducing ligand)-based ferroptotic agent therapy to be used in addition to conventional hyperthermic intraperitoneal chemotherapy to treat OPC patients after cytoreductive surgery.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Ferroptosis Apoptosis Synergistic interaction integration of death signals humanized patient-derived xenograft (PDX) mouse model tumoroids

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

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

*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

The specific aims of this project are to: (1) investigate the mechanism of the synergistic induction of cytotoxicity caused by the interplay between apoptosis and ferroptosis during the combined treatment, and (2) examine genetic signatures of tumor tissues from ovarian peritoneal carcinomatosis (OPC) patients and examine the preclinical efficacy of the multimodal approach (Fc-TRAIL + ferroptotic agent) in a humanized patient-derived xenograft (PDX) mouse model of OPC.

**Major Task 1: Identify mutation status and transcriptional signatures of genes (3-18 months)**

Subtask 1: Determine the status of biomarkers in OPC tumor tissues

**Major Task 2: Establish PDX tumors and determine the growth and regression of PDX tumors during multimodal treatment (3-20 months)**

Subtask 1: Establish PDX tumors with humanized mice

Subtask 2: Examine preclinical efficacy of combinatorial treatment on PDX tumors

Subtask 3: Statistical analysis of data

**Major Task 3: Investigate mechanism of synergistic interaction between ferroptosis and apoptosis (3-24 month)—50% completion in December 2022.**

Subtask 1 Establish OPC organoid cultures

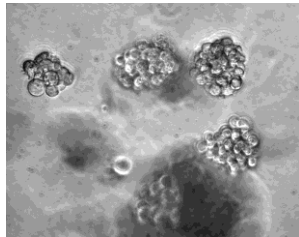
Subtask 2: Examine cytotoxicity of combinatorial treatment in organoid cultures

Subtask 3: Investigate interaction between ferroptosis and apoptosis in organoid cultures

**What was accomplished under these goals?** *For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings,*

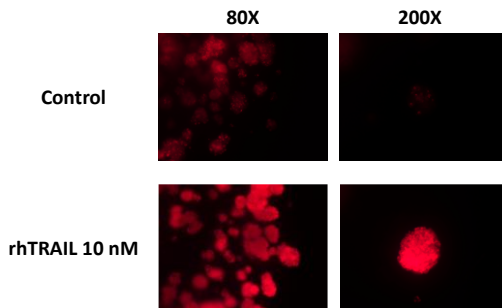
developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

**Establishment of organoid:** Tumor tissues from patients were used to establish 3 D culture. Tumor tissue fragments were washed and incubated in chelating solution supplemented with EDTA. After incubation, tissues were washed with basal culture medium and resuspended in Basement Membrane Matrix. The Basement Membrane Matrix was overlaid with Human Stem Cell medium.



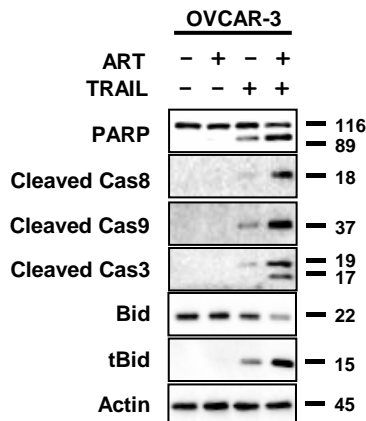
**Figure 1. Ex vivo primary organoid cultures**

**Cytotoxicity:** Organoids were treated with TRAIL and cytotoxicity was determined using PI staining assay as shown below. TRAIL induced cytotoxicity which was detected by DNA fragmentation..



**Figure 2. Cytotoxic effect of TRAIL on organoids.** Organoids were grown for 14 days in Matrigel and then treated with 10 nM rhTRAIL for 24 h. The organoids were stained with propidium iodide (PI) and fluorescent images were detected.

**Interaction between artesunate and TRAIL:** As shown in Figure 3, the combinatorial treatment of artesunate (ART) and TRAIL increased apoptosis (increased cleavage of PARP, caspases, and Bid).



**Figure 3. Detection of synergistic interaction between ART and TRAIL.** Ovarian OVCAR-3 cells were pretreated with ART (50  $\mu$ M) for 20 h and then exposed to human recombinant TRAIL (2 ng/ml) for an additional 4 h. Whole-cell extracts were analyzed with immunoblotting assay using indicated antibodies.

Ferroptosis is considered a distinctive form of cell death compared to other types of death such as apoptosis. It is known to result from iron-dependent accumulation of lipid peroxides rather than caspase activation. However, we observed that ferroptosis interplays with apoptosis in ovarian cancer cells. In this grant period, we investigated a possible mechanism of this interplay between ferroptosis and apoptosis. Results from our studies reveal that combined treatment of the ferroptotic agent artesunate and the apoptotic agent TRAIL effectively enhanced TRAIL-induced apoptosis by increased caspase activation. These results need to confirm in organoid culture.

*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

N/A

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

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

Nothing to report.

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

- (1) We will obtain tumor tissues from OPC patients and identify distinct mutation status and transcriptional signatures of genes (KRAS and TRAIL receptors). We will employ humanized triple transgenic NSG<sup>TM</sup>-SGM3 mice for establishing patient-derived xenograft (PDX) mouse models with KRAS mutant and TRAIL receptor-positive tumor tissues. We will then assess the preclinical efficacy of multimodal treatment (Fc-TRAIL + ferroptotic agent) on the growth and regression of PDX tumors.
- (2) We will examine the mechanism of interaction between ferroptosis and apoptosis. We hypothesize that an increased caspase activation is due to disruption of mitochondrial membrane potential ( $\Delta\Psi_m$ ). The alterations of mitochondrial membrane potential are probably due to an increase in oligomerization of BAX and its accumulation at the mitochondria during treatment with artesunate and TRAIL. This possibility will be investigated ovarian cancer organoids.

**4. IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

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

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

Nothing to report.

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

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

Nothing to report.

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

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

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

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Nothing to report.

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

*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

Nothing to report.

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

*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

Due to pandemic problems, lab had been closed and performance has been slow down.

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

*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

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

Nothing to report.

### Significant changes in use or care of vertebrate animals

Nothing to report.

### Significant changes in use of biohazards and/or select agents

Nothing to report.

**6. PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

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

*Report only the major publication(s) resulting from the work under this award.*

**Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Dilly AK, Honick BD, **Lee YJ**, Bartlett DL, Choudry HA. Rational application of targeted therapeutics in mucinous colon/appendix cancers with positive predictive factors. *Cancer Med.* 9:1753-1767, 2020. PMID 31958897
2. Lee YS, Kalimuthu K, Park YS, Makala H, Watkins SC, Choudry MHA, Bartlett, DL, Kwon YT, and **Lee YJ**. Ferroptotic Agent-induced Endoplasmic Reticulum Stress Response Plays a Pivotal Role in the Autophagic Process Outcome. *J Cell Physiol*, 2020;10.1002/jcp.29571 PMID 31985039
3. Lee YS, Kalimuthu K, Park YS, Choudry MHA, Bartlett, DL, and **Lee YJ**. BAX-dependent mitochondrial pathway mediates the crosstalk between ferroptosis and apoptosis. *Apoptosis*. 2020: doi: 10.1007/s10495-020-01627-z. PMID: 32737652

**Books or other non-periodical, one-time publications.** Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

**Other publications, conference papers and presentations.** Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (\*) if presentation produced a manuscript.

Nothing to report.

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

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**

*Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.*

Nothing to report.

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

*Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

Nothing to report.

- **Other Products**

*Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### What individuals have worked on the project?

*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.*

Example:

*Name: Mary Smith  
Project Role: Graduate Student  
Researcher Identifier (e.g. ORCID ID): 1234567  
Nearest person month worked: 5*

*Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.  
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Yong J. Lee—No change  
Name: Haroon Choudry—No change  
Name: Reetesh Pai—No change  
Name: Yongli Shuai—No change  
Name: Theresa Whiteside—No change  
Kalishwaralal Kalimuthu--postdoc fellow was terminated  
Jin Hong Kim—A new postdoc fellow is replaced for Kalishwaralal Kalimuthu  
Xiangwei Wu—No change

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

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

Nothing to report.

**What other organizations were involved as partners?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

*Provide the following information for each partnership:*

*Organization Name:*

*Location of Organization: (if foreign location list country)*

*Partner’s contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

A collaborator—Dr. Xu Luo, an Associate Professor at University of Nebraska, join as a collaborator.

## 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:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*