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TITLE: Overcoming Resistance to EGFR Inhibitors in Advanced Head and Neck Cancers

PRINCIPAL INVESTIGATOR: Barbara Burtness, Ph.D.

CONTRACTING ORGANIZATION: Yale University

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5e. TASK NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Head and neck squamous cell carcinomas (HNSCCs) affect more than a half million people annually worldwide. The goal of this proposal is to develop strategies to overcome HNSCC resistance to EGFR inhibitors (EGFRis). Based on extensive published and preliminary data, this proposal tests three hypotheses: 1) that upregulation of AURKA provides a major source of resistance to EGFRis in HPV- HNSCC. 2) that tumors most likely to upregulate AURKA will either have highly damaging LOF mutations in TP53, or upregulation of TPX2 or NEDD9, and that these tumors will have the highest level of resistance to EGFRis. 3) that targeting AURKA either with single agent kinase inhibitors, or in combination therapies that inhibit AURKA and a second protein, WEE1, that collaborates with AURKA to control mitotic progression, will be particularly effective in counteracting resistance to EGFRis. In the first year of funding, we have defined the pattern of TP53 mutations in HNSCCs. We have created HNSCC cell models resistant to EGFR-targeting inhibitors, and have been profiling response to AURKA and WEE1 inhibitors. We have accrued specimens from a clinical trial of EGFR inhibitors in HNSCC, which will support correlating therapeutic resistance with AURKA, TPX2, and NEDD9 expression. Work is ongoing.

15. SUBJECT TERMS
Head and neck cancer, head and neck squamous cell carcinoma, targeted therapy, TP53, AURKA, EGFR, WEE1, resistance, combination therapy.

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DOD Annual Report

Principal Investigator: Barbara Burtness, MD
Institution: Yale School of Medicine
Grant Number: W81XWH-21-1-0488

INTRODUCTION:

The goal of the project is to address the interactions between TP53 mutation status, and the utility of using inhibitors of AURKA, WEE1, and EGFR to achieve therapeutic benefit. Specifically, we proposed to determine how TP53 mutation class (damaging or benign) affected AURKA expression and sensitivity to inhibition of AURKA and EGFR. We also proposed to explore the relative efficacy of AURKA used alone, or an AURKA-WEE1 inhibitor combination, in EGFR inhibitor-resistant tumors, and to determine whether these drugs could preventing or reverse adaptive resistance to EGFR inhibition. Finally, we proposed to define the relationship between TP53 genotype, AURKA expression, and response to EGFR inhibition using clinical trial samples for HNSCC.

KEYWORDS:

Head and Neck Cancer, Head and neck squamous cell carcinoma, targeted therapy, TP53, AURKA, EGFR, WEE1, resistance, combination therapy.

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

What were the major goals of the project?

The goals of the project as approved in the SOW are as follows:

Specific Aim 1: Determine how TP53 mutation class determines AURKA expression and alters sensitivity to inhibition of AURKA and EGFR.

Specific Aim 2: Explore relative efficacy of AURKA monotherapy and an AURKA-WEE1 inhibitor combination in the setting of and in preventing or reversing adaptive resistance to EGFR inhibition.

Specific Aim 3: Define the relationship between TP53 genotype, AURKA expression, and response to EGFR inhibition using clinical trial samples for HNSCC.

What was accomplished under these goals?

Report on activity.
We are on track to achieve the goals of this project, based on progress over the first year. Work over this year primarily addressed Aims 1 and 2. Broken down by subtask specified in the Statement of Work, our data from this project are as follows:
Specific Aim 1: Determine how TP53 mutation class determines AURKA expression and alters sensitivity to inhibition of AURKA and EGFR.
Specific Aim 2: Explore relative efficacy of AURKA monotherapy and an AURKA-WEE1 inhibitor combination in the setting of and in preventing or reversing adaptive resistance to EGFR inhibition

Specific Aim 3: Define the relationship between TP53 genotype, AURKA expression, and response to EGFR inhibition using clinical trial samples for HNSCC**.**

Major task 1 (Aim 1), to be performed over months 1-6 was for the Golemis group to use public resources to establish relationship between TP53 mutation segregated by class, and expression of mRNA for AURKA, NEDD9, and TPX2. This work is substantially completed. As a first step in this analysis, we assembled a dataset of 794 patients with HPV-negative HNSCC for whom data were available in cBioportal from The Cancer Genome Atlas PanCancer and Broad Institute data sets, and from AACR-GENIE public resources. Within this dataset, TP53 mutations have been assigned into different functional classes (e.g., considered damaging based on ACMG criteria, Poeta criteria, or others). This classification analysis has been published, as part of a broader study of TP53 mutation patterns in HPV-negative head and neck squamous cell carcinoma, with DOD funding acknowledged (Deneka et al, Clin Cancer Res 2022; PMID 35491653).

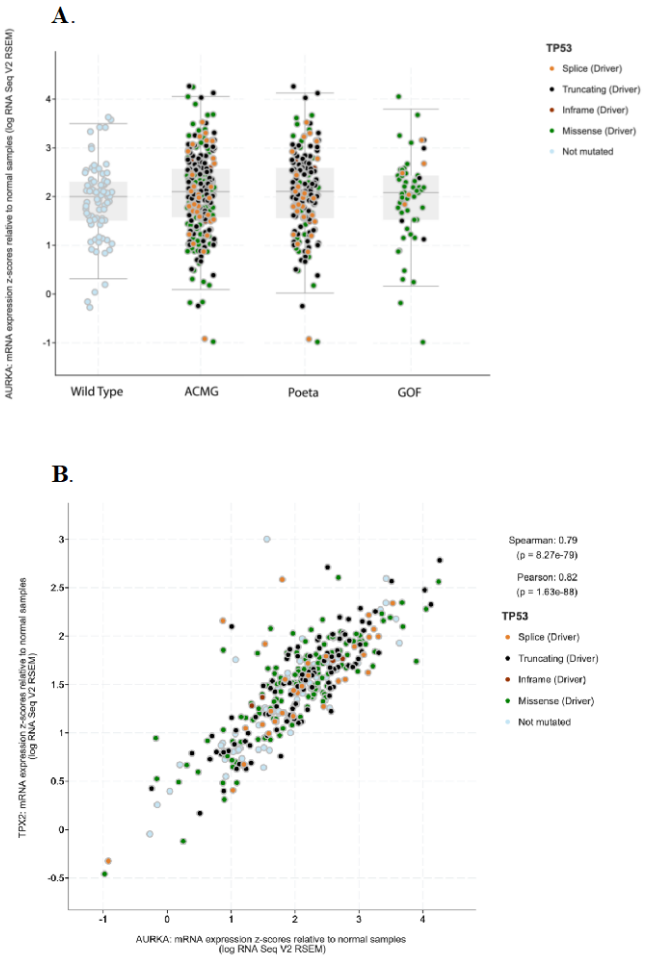

Figure 1. Expression of AURKA based on TP53 mutation class and TPX2 expression. **A**. AURKA expression does not significantly differ in HNSCC tumors bearing distinct classes of TP53 mutations. **B**. AURKA mRNA expression is highly correlated with expression of TPX2.

For specimens for which mRNA was available, we then analyzed levels of expression of AURKA, NEDD9, and TPX2, examining average level of expression in specimens with different classes of TP53 mutation, and also correlating expression of AURKA, NEDD9, and TPX2 with each other. Based on this, we found that while AURKA mRNA expression in HPV-negative HNSCC tumors was significantly elevated above that in normal adjacent tissue, as is well established (not shown), mRNA expression did not significantly differ based on whether tumors contained wt TP53, or TP53 mutations of various distinct biological classes, including ACMG criteria for pathogenic, Poeta classification for extremely damaging/pathogenic, or gain-of-function (**Figure 1A**). We also performed analysis to explore all potential correlations or between expression of TP53, AURKA, TPX2, and NEDD9. Of these, a very highly significant correlation was between AURKA and TPX2 mRNA expression (**Figure 1B**); all other genes showed no correlation (not shown). This result strongly suggests an underlying source of resistance to AURKA inhibitors in HNSCC may lie in the common co-expression of TPX2 with AURKA, given a TPX2-AURKA interaction is established to reduce effectiveness of AURKA-targeting inhibitors.

Major tasks 2 (Aim 1) and 3 (Aim 20, to be performed over months 6-14, are to functionally test the relation between *TP53* mutation, AURKA mRNA and protein expression and activity, and resistance to the EGFR

inhibitors afatinib and erlotinib. For this, the first step was for the Burtness group to generate cell models in FaDu, Cal27, A431, and Detroit 562 cell lines that were made resistant to these EGFR inhibitors. To date, erlotinib and afatinib lines have been generated in the FaDu and Cal27 models, based on gradual step-up in drug dosage over several months.

Data for the resistant cell lines, reflecting work from the Golemis and Burtness groups, are shown in **Figure 2**. For afatinib-resistant FaDu cells, this reflects a difference in IC50 of 8 nM versus >1 μM; for afatinib-resistant Cal27, 5.8 versus 11.4 μM; for erlotinib-resistant FaDu, 1.8 versus 7 μM; and for erlotinib-resistant Cal27, 3.8 versus 11.2 μM. The establishment of other cell lines resistant to EGFR inhibitors is still in progress, but nearly complete. The selection process has proven to take time because of the need to do very gradual increases in drug dosage to avoid killing the cells, and because of the need to address some unexpected issues that arose (e.g., the tendency of resistant cells to lose attachment to tissue culture plastic). To achieve uniformity of analysis across all cell models, we will perform Western analysis of baseline and EGFR-inhibitor-induced expression of AURKA, TPX2, NEDD9, EGFR, and sentinel effectors once all models are in hand, so they can be analyzed in parallel to achieve greater consistency of findings. This should occur within the next few months.

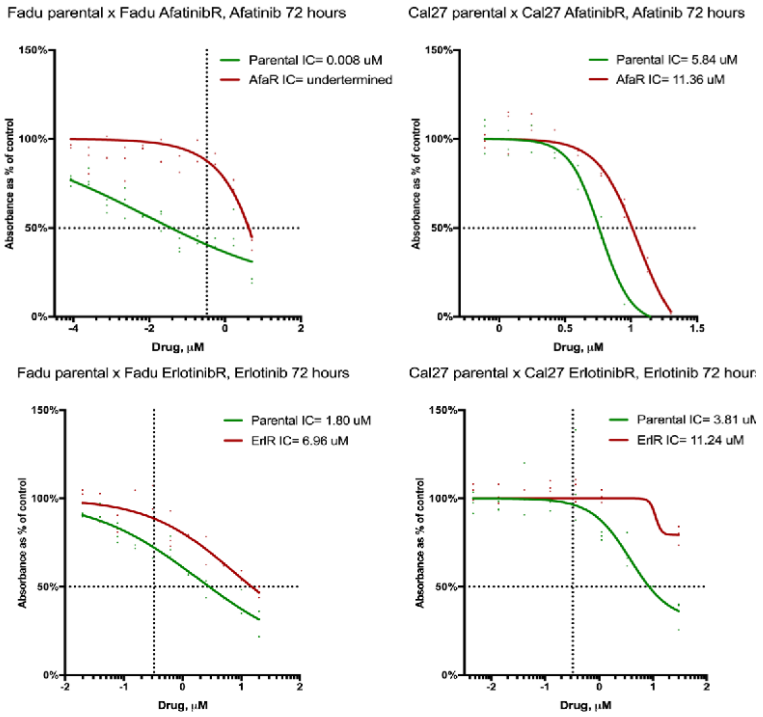

Figure 2. IC50 values of parental FaDu or Cal27 cells treated with erlotinib or afatinib, versus IC50 values of erlotinib- or afatinib-resistant derivatives, as indicated.

For Specific Aim 2, the goal is to explore relative efficacy of AURKA monotherapy and an AURKA-WEE1 inhibitor combination in the setting of and in preventing or reversing adaptive resistance to EGFR inhibition. Major task 3, to be addressed by both Burtness and Golemis laboratories over months 4-16 of the project, is to determine efficacy of AURKA inhibition with TAS-119 alone or in combination with adavosertib in afatinib- or cetuximab-resistant HNSCC. FaDu, Cal27, Detroit 562 cells and A431 cells. Techniques to be used include cell viability synergy assays, oncosphere, and anchorage independent growth assays, as well as functional assessment with flow cytometry and confocal microscopy. This is well under way with the existing cell models.

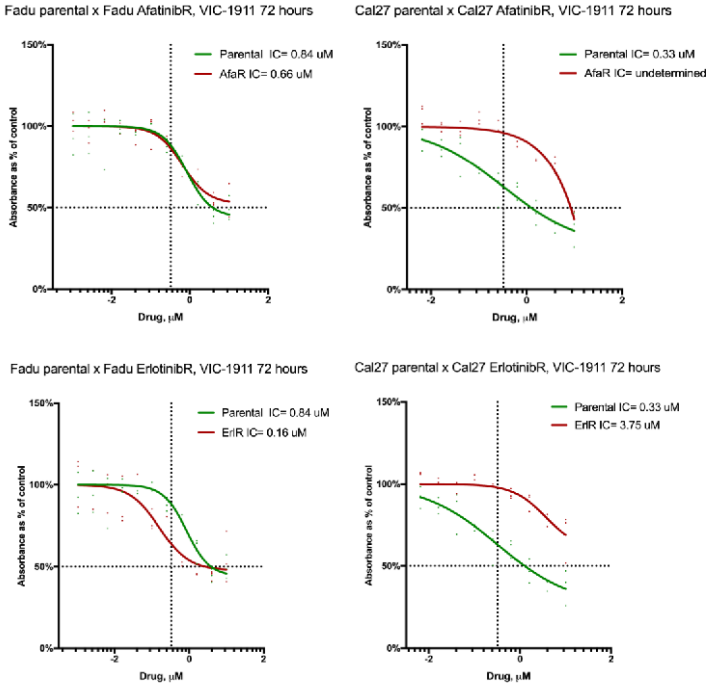

Figure 3. IC50 values of parental FaDu or Cal27 cells treated with VIC-1911, versus IC50 values of erlotinib- or afatinib-resistant derivatives, as indicated.

Examples of single agent IC50 data for treatment of resistant versus non-resistant lines with TAS-119 (renamed VIC-1911, due to purchase of the compound by another biotechnology company) or adavosertib include **Figures 3 and 4**. All data shown represent the average results from at least three independent experimental replicates.

This yielded somewhat surprising results, with resistance to EGFR targeting agents producing opposing outcomes for resistance to the AURKA inhibitor VIC-1911 in the FaDu versus the Cal27 cell models. In FaDu cells, afatinib or erlotinib resistance had no effect, or increased the sensitivity of cells to VIC-1911; the opposite was observed in Cal27 cells. In contrast, resistance to erlotinib or afatinib increased sensitivity to the WEE1 inhibitor adavosertib, with a modest effect in FaDu cells and a more significant effect in Cal27 cells.

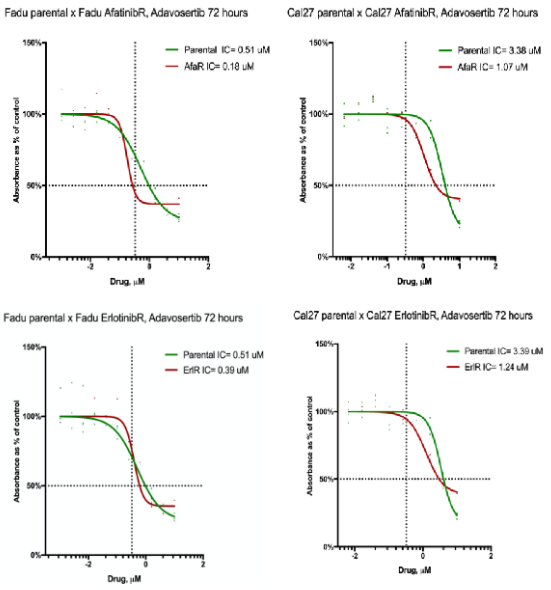

Figure 4. IC50 values of parental FaDu or Cal27 cells treated with adavosertib, versus IC50 values of erlotinib- or afatinib-resistant derivatives, as indicated.

We are in the process of performing synergy tests, using Loewe synergy plot analysis. Examples of analysis of synergy in the parental FaDu and CAL27 cell lines for the combinations of afatinib or erlotinib and VIC-1911, or adavosertib and VIC-1911, are shown in **Figures 5 and 6**. All data shown represent the average results from at least three independent experimental replicates.

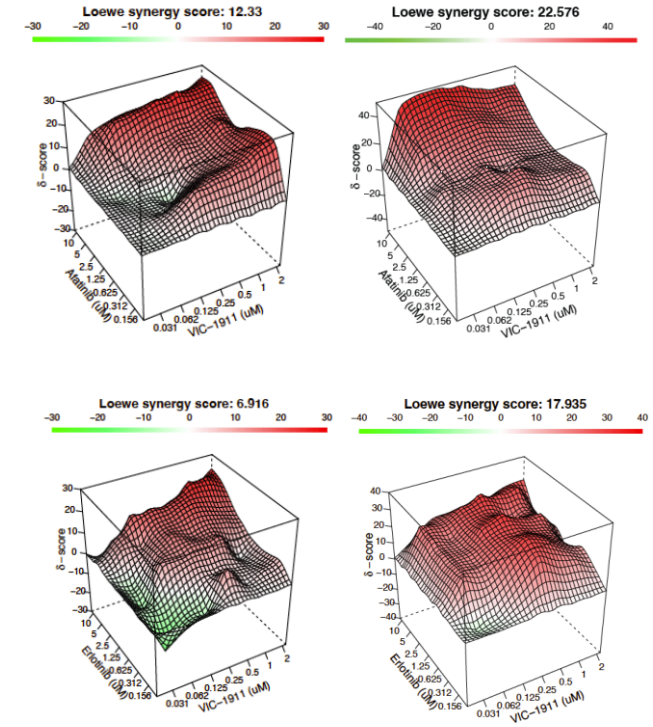

Figure 5. Loewe synergy score for combination of the indicated EGFR inhibitors with VIC-1911 at multiple ratios, as indicated. Left, FaDu; right, Cal27; top, afatinib; bottom, erlotinib.

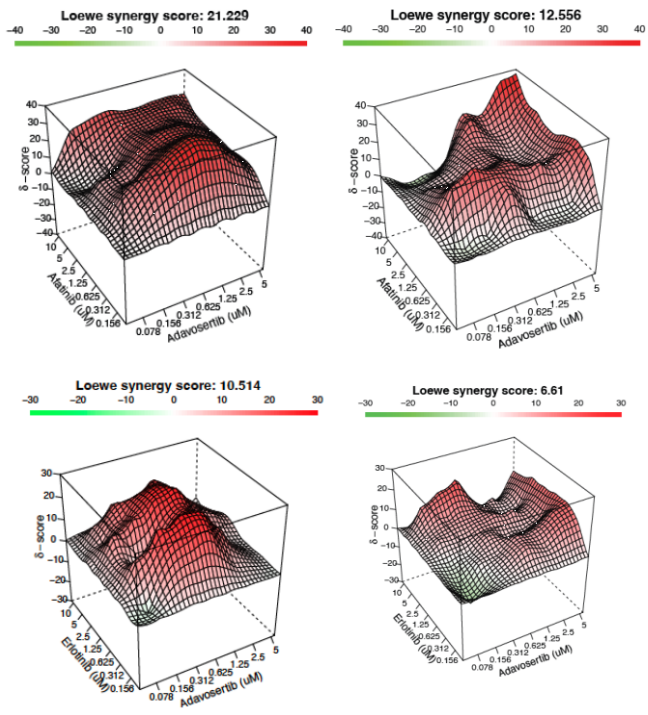

Figure 6. Loewe synergy score for combination of the indicated EGFR inhibitors with adavosertib at multiple ratios, as indicated. Left, FaDu; right, Cal27; top, afatinib; bottom, erlotinib.

Based on this analysis, the most synergistic drug ratios, and degree of synergy for each model are summarized here:

Cell Lines	Drug 1	Drug 2	Loewe synergy score	Most synergistic ratio (Drug 1/ Drug 2)	Optimal Ratio synergy score
Fadu	Afatinib	VIC-1911	12.33	1:3	16.3
	Erlotinib	VIC-1911	6.92	5:1	24.6
Cal27	Afatinib	VIC-1911	22.58	5:1	32.2
	Erlotinib	VIC-1911	17.93	20:1	31.2

Cell Lines	Drug 1	Drug 2	Loewe synergy score (overall)	Most synergistic ratio (Drug 1/ Drug 2)	Optimal Ratio synergy score
FaDu	Afatinib	Adavosertib	21.23	1:2	37.6
	Erlotinib	Adavosertib	10.51	1:1	27
CAL27	Afatinib	Adavosertib	12.56	1:16	28.8
	Erlotinib	Adavosertib	6.61	1:8	19.5

In Loewe analysis, synergy scores above 10 typically reflect synergy, while those below -10 reflect antagonism. Very little antagonism was observed at any concentration or combination ratio, whereas many concentrations and combination ratios demonstrated high levels of synergy. These preliminary data support the idea of using EGFR-targeting drugs in combination with VIC-1911 or adavosertib. Similar experiments are now in progress using the erlotinib and afatinib resistant lines. We are also using the optimal ratio of drugs selected from IC50 data to perform the additional assays (clonogenic, FACS, etc) noted in this aim, with this work in progress.
For Aim 2, major task 3, we are also preparing to perform specified xenograft experiments. For this purpose, the Burtness laboratory has successfully obtained ACURO approval for our animal protocol to perform the described in vivo xenograft experiments.
For Aim 3, Major task 6, the Burtness group will analyze specimens from an ongoing clinical trial of response and progression-free survival in patients treated on a phase II trial of afatinib and cetuximab for HNSCC (NCT02979977). Accrual of specimens to this trial is currently 39 of 50 patients. Accrual of 6 patients over the past year was slightly below the anticipated volume due to COVID-related staffing constraints, now resolved. We anticipate accruing the remaining 11 patients in the next 12 to 18 months. Baseline tissue is available from all patients. Post-treatment biopsies are in hand from 18 patients. A patient-derived xenograft from an on-study biopsy from an immunotherapy-experienced patient is currently growing. Based on this data, we are on track to perform the proposed experiments on the schedule described in the statement of work for this proposal.

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

Work on this project has provided training to a post-bac student in the Burtness lab, Sundong Kim.

How were the results disseminated to communities of interest?

Burtness and Golemis co-authored an original article related to this work.

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

We will continue progressing through the specific aims of the project, as specified in the SOW. We anticipate significant progress on aims assessing the interaction of drug resistant cell lines with treatment with AURKA and WEE1 inihibitors.

IMPACT:

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

The Clinical Cancer Research paper by Deneka et al published in 2022 was the first detailed analysis of the profile of TP53 mutations in HNSCC, and their relationship to tumor mutation burden and disease subsite, among other parameters. This provides an important baseline of information for studies of HNSCC involving use of agents expected to cause cell cycle abnormalities that can lead to aneuploidy, such as treatment with WEE1 and AURKA inhibitors (the topic of this proposal) – but also other agents.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

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

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

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

None

Significant changes in use or care of human subjects

Significant changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents

PRODUCTS:

Publications, conference papers, and presentations

Deneka, A.Y., Baca, Y., Serebriiskii, I.G., Nicolas, Parker, M.I., Nguyen, T.T., Korn, W.M., Demeure M.J., Wise-Draper, T., Sukari, A., E., Burtness, B., and Golemis, E.A. Association of TP53 and CDKN2A mutation profile with tumor mutation burden (TMB) in head and neck cancer. Clin Cancer Res 2022 May 2;28(9):1925-1937; PMID: 35491653 PMCID: PMC9186806. Acknowledges Federal Support

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Barbara Burtness, MD*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-4660-1859*
Nearest person month worked:	*1*
Contribution to Project:	*Provided Direction*
Funding Support:	*DOD*
Name:	*Jong Woo Lee, PhD*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-8352-4269*
Nearest person month worked:	*1*
Contribution to Project:	*Conducted experiments*
Funding Support:	*DOD*

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

Please see attached Other Support document for Drs. Burtness and Lee.

What other organizations were involved as partners? None.

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.

SPECIAL REPORTING REQUIREMENTS

Not applicable.

COLLABORATIVE AWARDS:

QUAD CHARTS:

APPENDICES:

Publications