**AWARD NUMBER:** **W81XWH-21-1-0488**

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

**PRINCIPAL INVESTIGATOR:** **Barbara Burtness, Ph.D.**

**CONTRACTING ORGANIZATION:** **Yale University, New Haven, CT**

**REPORT DATE:** **July 2023**

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

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**5b. GRANT NUMBER**

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Yale School of Medicine
333 Cedar Street
New Haven, CT
06520-8028

**8. PERFORMING ORGANIZATION REPORT 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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## INTRODUCTION:

Head and neck squamous cell carcinomas (HNSCCs) are frequently devastating cancers that affect more than a half million people annually worldwide. HNSCC is typically detected at an advanced stage, and when recurrent is treated with cytotoxic systemic therapies. The only approved targeted therapies for advanced disease are inhibitors of the epidermal growth factor receptor (EGFR), which improve disease outcomes in some patients. The goal of this proposal is to develop strategies to overcome intrinsic and acquired resistance to EGFR inhibitors (EGFRis). Aurora-A kinase (AURKA) upregulation is common in HPV- HNSCC, and activates critical downstream effectors of EGFR, including ERK1/2, AKT, and others; in addition, overexpressed AURKA causes mitotic abnormalities that promote genomic instability, leading to selection of resistant clones. Transcriptional upregulation of AURKA-binding proteins including TPX2 and NEDD9 protect AURKA from proteolytic degradation, enhancing its activity; upregulation of TPX2 and NEDD9 is common in advanced cancers, and overexpression of TPX2 has been shown to promote resistance to EGFRis. This proposal tests the hypothesis that upregulation of AURKA provides a major source of resistance to EGFRis in HPV- HNSCC. We hypothesize 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. We hypothesize 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.

**KEYWORDS:** EGFR, AURKA, WEE1, NEDD9, TPX2, TP53, alisertib, erlotinib, afatinib, adavosertib, synergy, resistance, head and neck cancer

## ACCOMPLISHMENTS:

### 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?

Aim 1, Major task 1 (use public resources to establish relationship between TP53 mutation segregated by class, and expression of mRNA for AURKA, NEDD9, and TPX2) was completed and described at the time of the last report and published in 2022.

Aim 1, Major task 2 (Functionally testing the relation between *TP53* mutation class, AURKA mRNA and protein expression and activity, and resistance to the EGFR inhibitors afatinib and erlotinib), HNSCC cell lines were developed by growth in gradually increasing concentrations of these drugs (Burtness group, year 1, reported previously), or by overexpressing the oncogene c-MET, a common physiological cause of resistance to EGFR-

inhibiting agents (Golemis group, this report period; see Figures 1-3). We are characterizing the expression of AURKA, TPX2, NEDD9, EGFR, and sentinel effectors in these models, and characterizing their cell cycle profiles to provide context for analysis of AURKA function (Figure 4-5, and work ongoing).

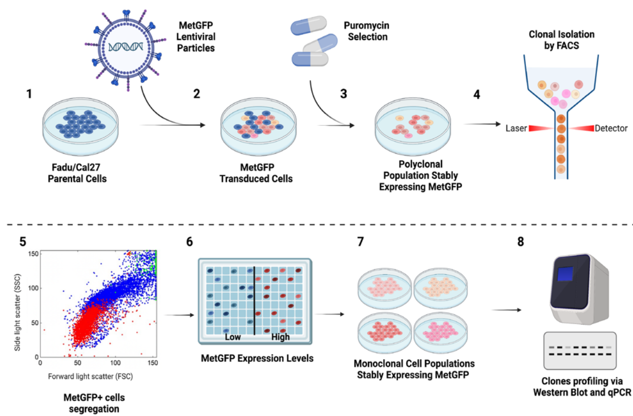
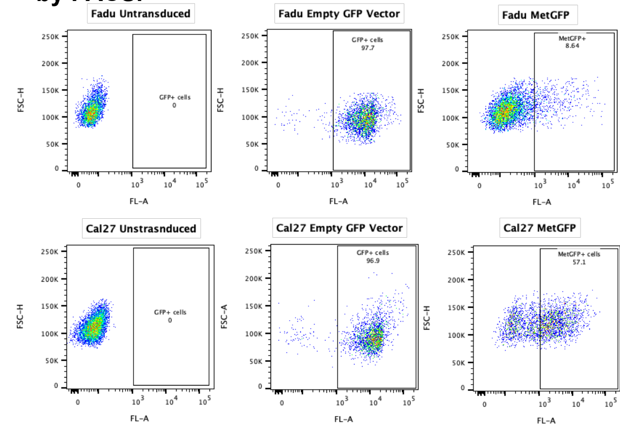



**Figure 1. Generation of Fadu and Cal27 with constitutive overexpression of c-MET. Schematic of steps employed to**

**Figure 2. Clonal isolation as part of development of c-MET overexpressing models; sorting of GFP+ Cells by FACS.**



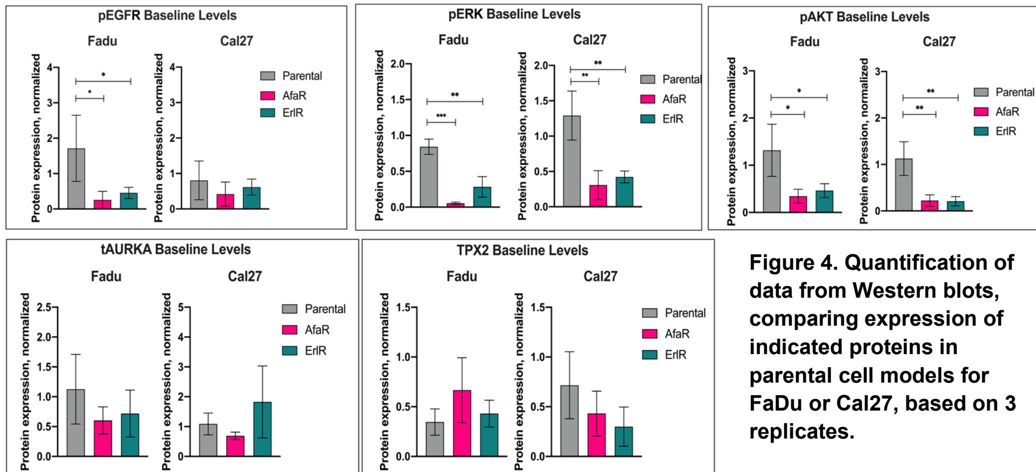




**Figure 4. Quantification of data from Western blots, comparing expression of indicated proteins in parental cell models for FaDu or Cal27, based on 3 replicates.**

We demonstrated AURKA expression by Western blot to be elevated in *TP53* mutated and null cells, relative to WT *TP53* cells. Employing a suite of PCI-13 engineered with wild type (WT), loss of function (LOF) and gain of function (GOF) mutations, we demonstrated enhanced sensitivity to AURKA inhibition.

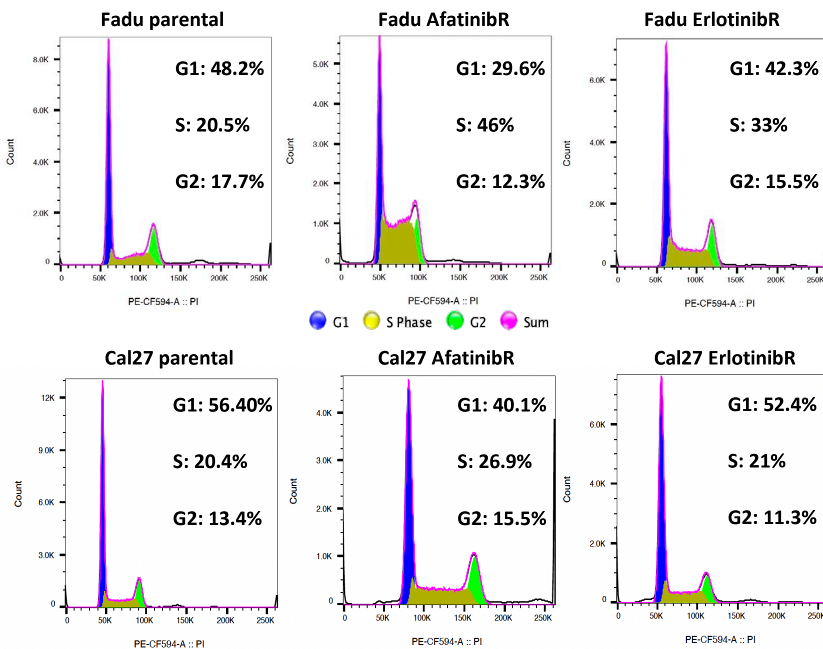



**Figure 5. Representative data from FACS analysis of asynchronously growing cell populations of FaDu or Cal27 cells, or Afatinib- or Erlotinib-resistant derivative lines, typical of 3 replicates. Resistance is associated with cell cycle defects.**

Aim 2, Major task 3 (Using squamous cell carcinoma lines with acquired resistance to cetuximab or afatinib, conduct cell viability, clonogenic survival, and xenograft modeling to determine the cytotoxicity of AURKA inhibition alone or in combination with WEE1 inhibition in HNSCC resistant to EGFR inhibition). Also, Aim 2, Major task 4 (Determine if AURKA inhibition or AURKA plus WEE1 inhibition restores sensitivity to EGFR inhibition in resistant models). As a major component of work in the past year, the Golemis group has been systematically assessing response of afatinib- and erlotinib-resistant cell lines

to the AURKA inhibitor TAS-119/VIC1911 and adavosertib, alone and in combination, as well as the combination of AURKA and EGFR inhibition, in the cell lines developed in year 1, as described in subtask 2, and while continuing to monitor the expression of AURKA, TPX2, NEDD9, EGFR, and sentinel effectors. Examples of these experiments are shown in Figures 6-13.

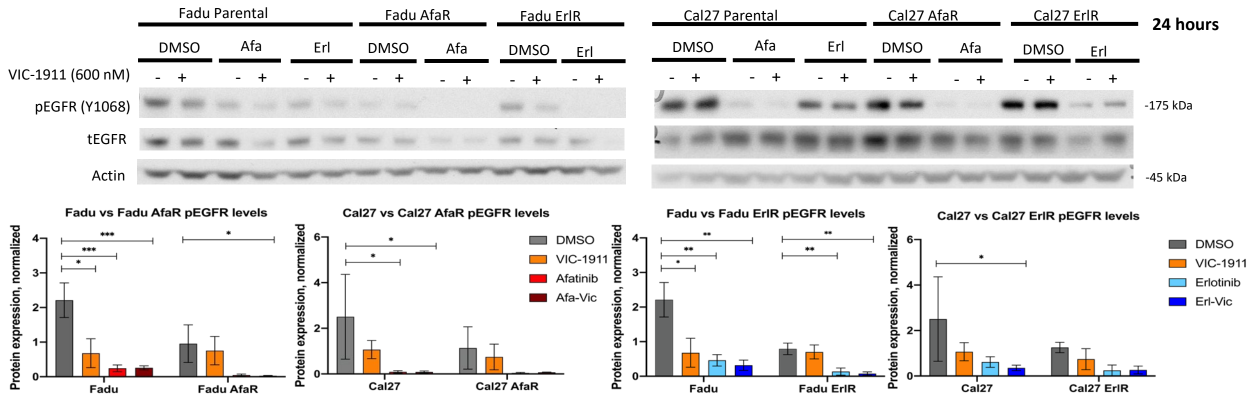


**Figure 6. Top, representative Western blot data and bottom, quantification from three replicates normalized to loading control, for expression of Y1068-phosphorylated EGFR from parental, AfaR, or ErlR resistant derivatives treated with the drugs indicated. *, p<0.05; **, p<0.01, ***. P<0.001.**

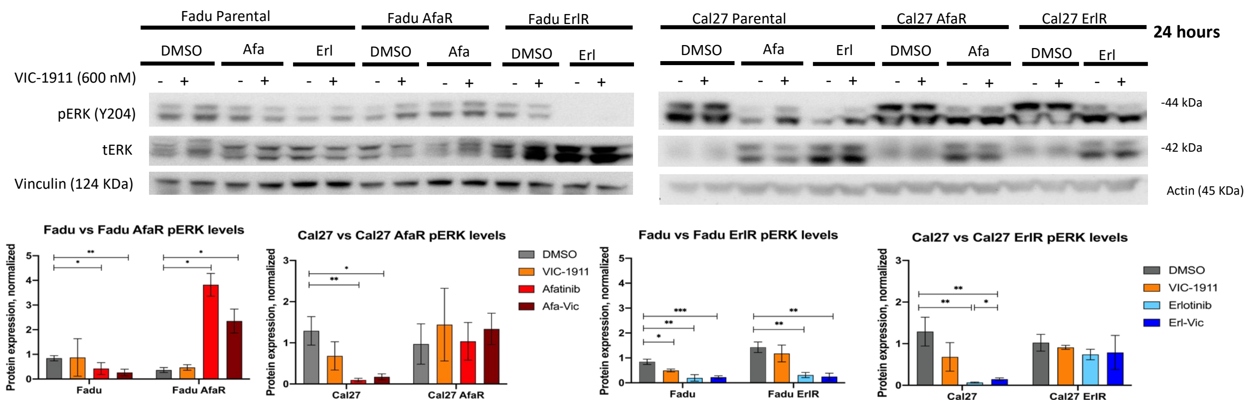


**Figure 7. Top, representative Western blot data and bottom, quantification from three replicates normalized to loading control, for expression of phosphorylated ERK1/2 from parental, AfaR, or ErlR resistant derivatives treated with the drugs indicated. *, p<0.05; **, p<0.01, ***. P<0.001.**

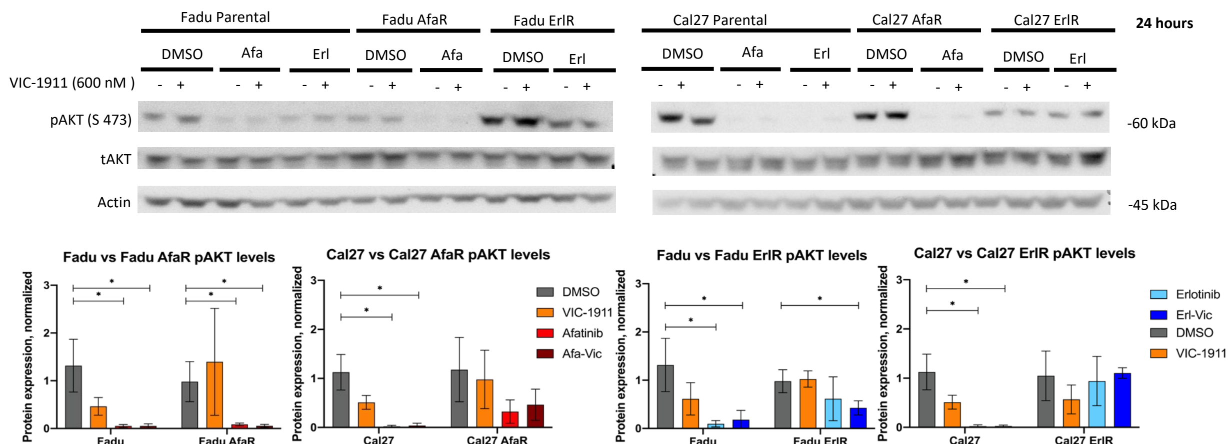


**Figure 8. Top, representative Western blot data and bottom, quantification from three replicates normalized to loading control, for expression of S473-phosphorylated AKT1 from parental, AfaR, or ErlR resistant derivatives treated with the drugs indicated. *, p<0.05; **, p<0.01, ***. P<0.001.**

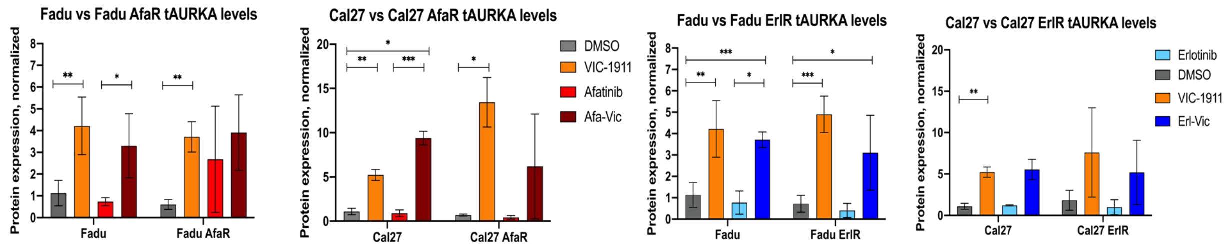



**Figure 9. Top, representative Western blot data and bottom, quantification from three replicates normalized to loading control, for expression of AURKA from parental, AfaR, or ErlR resistant derivatives treated with the drugs indicated. *, p<0.05; **, p<0.01, ***. P<0.001.**

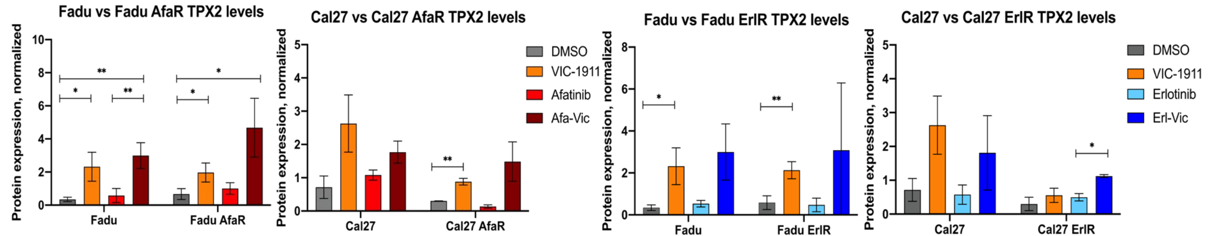
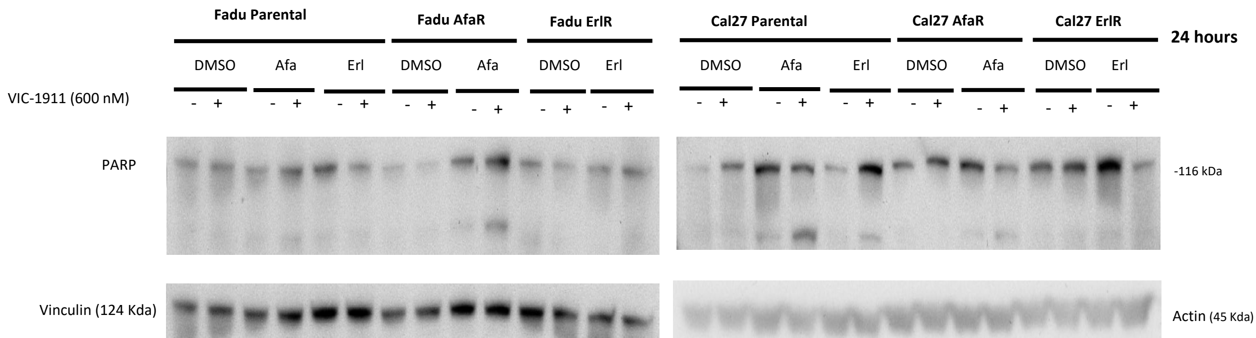



**Figure 10. Top, representative Western blot data and bottom, quantification from three replicates normalized to loading control, for expression of TPX2 from parental, AfaR, or ErlR resistant derivatives treated with the drugs indicated. *, p<0.05; **, p<0.01, ***. P<0.001.**




Fadu vs Fadu AfaR cleaved PARP

Cal27 vs Cal27 AfaR cleaved PARP

Fadu vs Fadu ErlR cleaved PARP

Cal27 vs Cal27 ErlR cleaved PARP

Protein expression, normalized

Fadu, Fadu AfaR, Cal27, Cal27 AfaR, Fadu ErlR, Cal27 ErlR

DMSO, VIC-1911, Afatinib, Afa-Vic, Erlotinib, Erl-Vic

**Figure 11. Top, representative Western blot data and bottom, quantification from three replicates normalized to loading control, for expression of PARP1 from parental, AfaR, or ErlR resistant derivatives treated with the drugs indicated. *, p<0.05; **, p<0.01, ***. P<0.001.**

**Figure 12. Left, representative FACSt data and right, quantification from three replicates, for expression of PARP1 from parental, AfaR, or ErlR resistant derivatives treated with the drugs indicated. *, p<0.05; **, p<0.01, ***. P<0.001.**

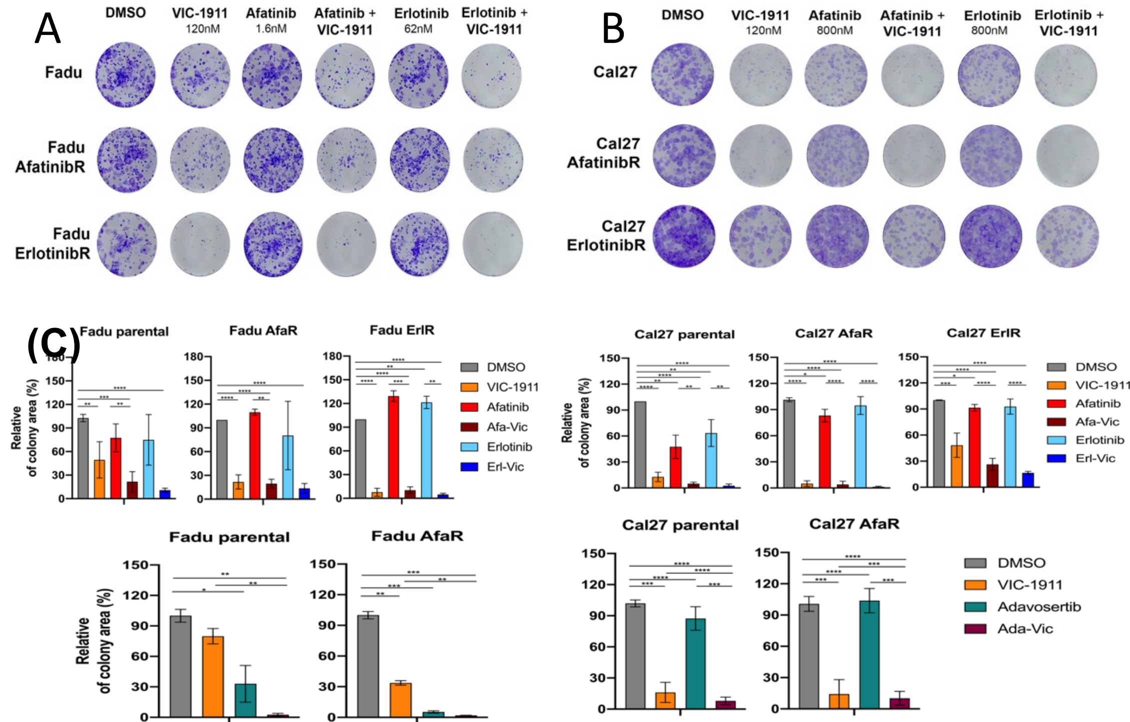



Figure 13. Clonogenic survival assays. A, B. Top, crystal violet staining of (A) Fadu and (B) Cal27 EGFR inhibitor -sensitive and –resistant cell lines after 12 days of treatment with DMSO or the indicated drugs. Images are representative of three biological three replicates. Cells were treated with DMSO, VIC-1911 (120nM), afatinib (1.6nM for Fadu and 800nM for Cal27), erlotinib (62nM for Fadu and 800nM for Cal27), or combination. Bottom, quantified clonogenic survival assay, n = 3. C. Quantified clonogenic survival assay. Cells were treated with DMSO, adavosertib (100nM for Fadu and 500nM for Cal27), VIC-1911 (50nM), or combination for 12 days. n = 2. P values are based on multiple t test comparisons. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001.

As a summary of specific findings from these in vitro experiments over the second reporting period, we have almost completed cell line generation suitable for profiling of the relationship between EGFR inhibitor resistance, AURKA, and WEE1. Cell lines selected for resistance to EGFR inhibition have reduced levels of active

(phosphorylated) EGFR, ERK, and AKT, suggesting reduced dependence on this signaling. However, they do not upregulate AURKA and TPX2, and they have evidence of altered cell cycle. These cells retain sensitivity to AURKA inhibition, and downregulate EGFR activation further in response to treatment with inhibitors of EGFR and AURKA; however, the consequence of treatment with these inhibitors on the activation of ERK1/2 and AKT1 is variable between distinct resistant cell line models. AURKA inhibition typically leads to increased expression of AURKA, coupled with elevated expression of the AURKA-stabilizing protein TPX2. In both direct measures of apoptosis and in clonogenic assays, the adavosertib-VIC1911/TAS119 drug combination retains efficacy in synergistically promoting the death of FaDu cells that are resistant to EGFR inhibitors; CAL27-derived EGFR-resistant cells are less responsive to AURKA inhibition, although adavosertib remains effective in inducing cell death.

The Burtness lab developed EGFR-resistant models by continuous culture in increasing doses of erlotinib or afatinib. Xenografts were established from parental or afatinib-resistant FaDu cells subcutaneously injected into dorsal flanks in 7 to 8-week-old athymic female mice. Tumor-bearing mice were treated with vehicle, adavosertib (120mg/kg, q.d., p.o.), VIC-1911 (30 mg/kg, q.d., p.o.) or combination for 21 days once tumors reached ~150-250 $mm^3$. Compared to parental FaDu tumors, afatinib-resistant xenografted tumors demonstrated significantly greater tumor control with VIC-1911 monotherapy (Fig. 14). Furthermore, the combination of VIC-1911 and the WEE1 inhibitor adavosertib was synergistic in both parental and afatinib-resistant tumors, with greater effects in the resistant model (Fig. 15).

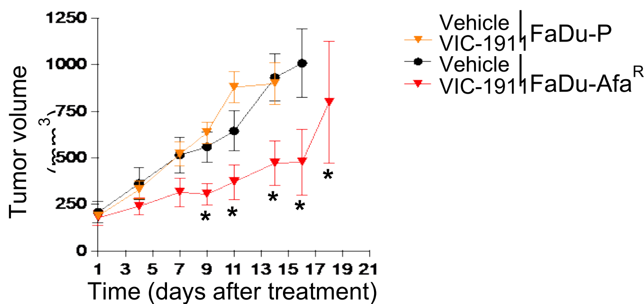



**Fig 14. Afatinib-resistant HNSCC xenografted tumor is sensitive to AURKA inhibitor VIC-1911 *in vivo*. Mice harboring either FaDu parental (FaDu-P) or afatinib-resistant (FaDu-Afa$^R$) tumors were daily treated with vehicle (n=7) or VIC-1911 30 mg/kg (n=8) for 18 days.**

***, *P* < 0.05; ** *P* < 0.005. Data are shown as mean ± SEM.**

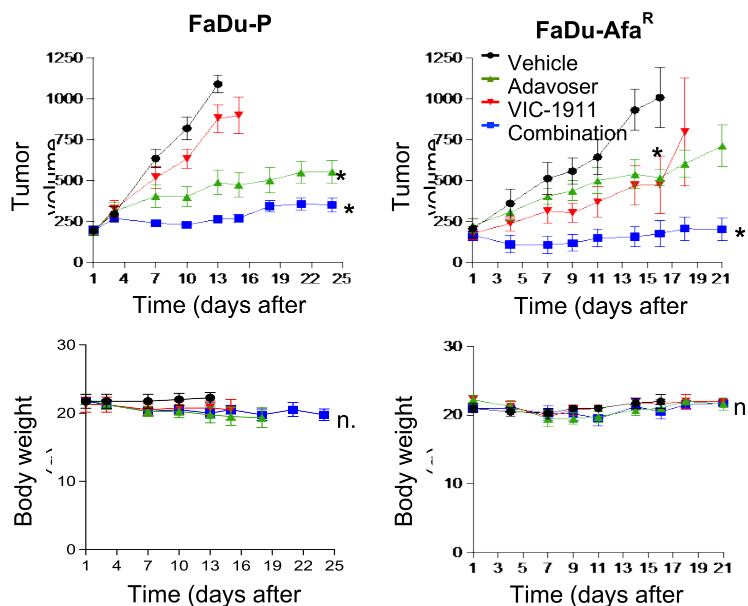



**Fig 15. Combination AURKA/WEE1 inhibitor treatment is effective against acquired resistant HNSCC tumor to EGFR inhibitor *in vivo*. Mice bearing either FaDu-P or FaDu-Afa$^R$ subcutaneous xenograft tumors were daily treated with vehicle (n=7), adavosertib 120 mg/kg (n=7), VIC-1911 30 mg/kg (n=8), or combination (n=8) for 21 days. n.s., not significant; *, *P* < 0.05; **, *P* < 0.005. Data are shown as mean ± SEM.**

Specific Aim 3. The clinical trial continues to accrue well (Burtness group), with accrual currently at 46 of the 50 planned patients. No new or unexpected safety signals have been observed. Sample collection is proceeding as

projected. One patient-derived xenograft (PDX) has been developed from a baseline biopsy. We anticipate completing accrual within the next 6 months. All regulatory approvals are up to date and have been received at DOD.

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

The Burtness lab participated in the Yale Cancer Center Introduction to Cancer Research Program in collaboration with Albertus Magnus, a minority-serving institution in New Haven, CT. Mr. Julian Barrantes, a talented intern who worked in the Burtness lab supporting the animal experiments for this project as part of his internship assignment in this program, has joined the Burtness lab as a post-baccalaureate student. Mr. Barrantes is a first-generation college graduate from a low income Hispanic community in Bridgeport, CT. Throughout his time in college he worked to care for his younger siblings and to contribute to the family income. His aspiration is to attend medical school or graduate school and become a cancer researcher.

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

One article was published. In addition, results from the study were presented in a poster at the American Association of Cancer Research annual meeting in 2023, as well as at the annual research days at Fox Chase Cancer Center and at Drexel University School of Medicine.

**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.

**IMPACT:**

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

Nothing to Report.

**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**

Nothing to Report.

**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:** Nothing to Report.

**Publications, conference papers, and presentations**

**Journal publications.**

This article was accepted but not published at the time of the last progress report – full citation appears here:

Nguyen, T.T., Silva, F.N., and Golemis, E.A. Aurora kinases in head and neck cancer. Cancer J. 2022 Sep-Oct 01;28(5):387-400. doi: 10.1097/PPO.0000000000000614. PMID: 36165728 PMC9836054. Federal support acknowledged.

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

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

AURKA inhibition influences glycolytic and hypoxic signaling in head and neck cancer. Nguyen, T.T., Silva, F.N., Lee, J.W., Burtness, B., and Golemis, E.A. Presented as poster at Drexel College of Medicine Discovery Day, October, 2022. Federal support acknowledged.

Synthetic lethal targeting of AURKA in Head and Neck Squamous Cell Carcinoma. Silva, F. N., Nguyen, T.T., Lee, J.W., Burtness, B., and Golemis, E.A. Presented as poster #542, AACR Annual Meeting, April 2023. Federal support acknowledged.

AURKA inhibition as a means of overcoming resistance to EGFR inhibitors in head and neck cacer. Silva, F. N., Nguyen, T.T., Lee, J.W., Burtness, B., and Golemis, E.A. Poster Presentation, Fox Chase Cancer Center Research Day, June 2023. Federal support acknowledged.

Concomitant inhibition of Aurora kinase A and WEE1 kinases results in synergistic tumor control and heightens DNA replication stress in head and neck and lung carcinomas. Lee JW, Kim S, Cruz Gomez S, Shi J, Yang C, Burtness B. Abstract 1563: *Cancer Res* (2023) 83 (7_Supplement): 1563.

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

https://www.aacr.org/wp-content/uploads/2023/04/AACR2023_AbstractPresentations040123.pdf

**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: | *PI, Professor* |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | *1* |
| Contribution to Project: | *Dr. Burtness has overall responsibility for research design and conduct at Yale.* |
| Funding Support: | *See Other Support document* |
| **Name:** | ***Jong Woo Lee, PhD*** |
| Project Role: | *Research Scientist* |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | *2 months* |
| Contribution to Project: | *Dr. Lee performed the experiments at Yale, and provided day to day in lab supervision of other personnel.* |
| Funding Support: | *See Other Support document* |
| **Name:** | ***Sundong Kim*** |
| Project Role: | *Post-baccalaureate trainee* |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | *1* |
| Contribution to Project: | *Mr. Kim performed experiments and helped maintain animals.* |
| Funding Support: | *See Other Support document* |
| **Name:** | ***Julian Barrantes*** |
| Project Role: | *Post-baccalaureate trainee* |

| Researcher Identifier (e.g. ORCID ID): | |
|---|---|
| Nearest person month worked: | *1* |
| Contribution to Project: | *Mr. Barrantes performed experiments and helped maintain animals.* |
| Funding Support: | *See Other Support document* |

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

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

**Organization Name:** Fox Chase Cancer Center

**Location of Organization:** Philadelphia PA

**Partner's contribution to the projec**t *(identify one or more)*

**Financial support;** Nothing to report

**In-kind support** Nothing to report

**Facilities** Nothing to report

**Collaboration** Yes, MultiPI collaborator on project

**Personnel exchanges** Nothing to report

**Other.** Nothing to report

**SPECIAL REPORTING REQUIREMENTS**

**COLLABORATIVE AWARDS:** See above

**QUAD CHARTS:**

**APPENDICES:** None

**SUPPORT**
**BURTNESS, BARBARA**

**Current**

**Title:** ECOG-ACRIN Cancer Research Group
**Time Commitments:** .54 calendar
**Supporting Agency:** National Cancer Institute 5UG1CA189828-09
**Address:** 37 Convent Dr. Bethesda, MD 20814
**Contracting/ Grants Officer:** Chris Schleyer
**Performance Period:** 08/01/2022-07/31/2023
**Level of Funding:**
**Project Goals:** The Head and Neck Committee seeks to improve outcomes for patients with all stages of head and neck cancer
**Specific Aims:** The Specific Aims are to conduct clinical trials of novel therapies and therapeutics strategies for patients with head and neck cancer.
**Overlap:** None

**Title:** Yale Head and Neck Cancer SPORE: Overcoming Treatment Resistance in Head and Neck Cancer **Time Commitments:** 2.4 calendar
**Supporting Agency:** NIH/NCI/NIDCR 1P50DE030707 Burtness (PI)
**Address:** 37 Convent Dr. Bethesda, MD
**Contracting/ Grants Officer:** April Harrison
**Performance Period:** 09/22/2020-6/30/2025
**Level of Funding:**
**Project Goals:** The Yale Head and Neck SPORE is a team of clinical and laboratory researchers studying the problem of treatment resistance in head and neck cancer. Each of three projects brings a basic and clinical research leader together to study ways to overcome resistance to common therapies, such as cetuximab and cisplatin, as well as to find ways to make HPV-driven cancers more sensitive to immunotherapy. There are clinical trials to treat patients with HPV-negative and HPV-driven cancer before they go to surgery. The projects are supported by core facilities to provide administrative, pathology and statistical support, and there are funds to support new projects and new investigators.
**Specific Aims:** Aim 1: To overcome resistance to EGFR inhibition in HNSCC by targeting active conformations of ErbB family members; Aim 2: To advance rational synthetic lethal combination therapy to the clinic in HPV-negative HNSCC; Aim 3: To advance combination demethylating therapy with immune checkpoint inhibition to the clinic for HPV-mediated HNSCC, with mechanistic studies and characterization of immune response; Aim 4: To bolster the foundation for HNSCC research through our Administrative, Biospecimen and Biostatistics/Bioinformatics cores, to engage institutional resources and the wider SPORE community; and Aim 5: To advance new research and to foster the next generation of HNSCC translational researchers through a Developmental Research Program, a Career Enhancement Program, and interaction and collaboration with the wider SPORE and HNSCC research communities.
**Overlap:** None

**Title:** Overcoming resistance to EGFR inhibitors in advanced head and neck cancers Translational Team Science Award
**Time Commitments:** 0.9 calendar
**Supporting Agency:** Department of Defense (W81XWH2110488) Burtness/Golemis (PI)
**Address:** USA MED Research ACQ Activity 820 Chandler St, Fort Detrick, MD 21702-5014
**Contracting/Grants Officer:** Amanda Carrera

**Performance Period:** 07/01/2021-06/30/2026
**Level of Funding**:
**Project Goals:** The goal of this project is to correlative *TP53* mutation class with AURKA expression and sensitivity to AURKA and EGFR inhibition, study AURKA inhibitors and inhibitor-based combinations in preventing and overcoming adaptive resistance to EGFR inhibitors, and to validate findings in specimens from an ongoing trial of dual EGFR blockade.
**Specific Aims:** *Aim 1: TP53* mutation class determines AURKA expression and is a determinant of sensitivity to inhibition of AURKA and EGFR. *Aim 2*: Inhibition of AURKA in combination with inhibition of WEE1 to overcome resistance to EGFR inhibition. *Aim 3*: AURKA and AURKA-associated proteins as biomarkers of response to EGFR inhibition, using specimens from a clinical trial of EGFRis for HNSCC.
**Overlap**: None

**Title:** Therapy for FA and HPV- related Head and Neck Cancers
**Time Commitments:** 2.4 calendar
**Supporting Agency:** Stand Up To Cancer (SU2C) Burtness (PI)
**Address:** 10880 Wilshire Blvd. Suite 1400 Los Angeles, CA 90024
**Contracting/Grants Officer:** Stacy J. Gillenwater
**Performance Period:** 10/01-2021-09/30/2024
**Level of Funding**:
**Project Goals:** The goal of our study is to develop improved treatments for patients with head and neck cancer. We will concentrate on treatments for patients who develop this cancer due to infection with a common virus called human papillomavirus (HPV) or those who have inherited a disease called Fanconi anemia, which predisposes them to this type of cancer at an early age. Cancers that develop due to HPV infection, are often diagnosed when cancer has already spread, and a quarter of these patients are not cured with current therapies. Standard therapies cannot be used in patients with Fanconi anemia due to their disease, leading to poor survival of Fanconi anemia patients who develop cancer. In both patient groups, successful treatment may be associated with terrible side effects resulting in low quality of life. For these reasons, it is imperative to identify new treatment and preventive strategies. We believe that our research will be helpful to all patients with head and neck cancer.
**Specific Aims:** In the work proposed here, we will test four major hypotheses.
**1.** By improved understanding of HPV-related and Fanconi anemia-associated cancers, we will identify actionable tumor-specific vulnerabilities which, with the assistance of novel cellular and engineered animal models, will direct us to new, safer, and more effective prevention and treatment strategies for HNSCC in these populations.
**2.** Combinatorial therapeutic approaches are safer and more efficacious in both HPV-related and FA-associated HSCCs, and these combinations may be cohort-specific.
**3.** Tumorigenesis can be inhibited in the FA-associated HNSCC using chemoprevention and in vivo gene therapy
**4.** Toxicity of the potential treatments of FA cancer patients may be successfully assessed using bone marrow function of the FA mouse model.
**Overlap**: None

**Title:** Synthetic Lethal Approaches to Treatment of FA Gene Mutant Head and Neck Cancer
**Time Commitments:** 0.12 calendar
**Supporting Agency:** Fanconi Anemia Research Foundation Burtness (PI)
**Address:** 1801 Willamette Street, Suite 200 Eugene, Oregon 97401
**Contracting/Grants Officer:** Laura Hefner
**PerformancPeriod:**09/01/2020-12/31/23
**Level of Funding**:
**Project Goals:** The goal of this project is to develop models of Fanconi gene-driven head and neck cancer

from sporadic head and neck cancers with Fanconi gene abnormalities, in order to test the proposition that cell cycle-active combination therapies provide an effective anticancer treatment for Fanconi Anemia-related head and neck cancer, while avoiding the intolerable toxicities of DNA-damaging therapies in FA patients.
**Specific Aims:** Aim 1: Identify HPV-negative HNSCC with sporadic FA gene abnormalities as a resource for studying FA-associated HNSCC
Aim 2: Develop pre-clinical models of FA-related HNSCC
Aim 3: Examine PLK1, WEE1, and AURKA inhibition in the FA pathway.
**Overlap**: No scientific or budgetary overlap

**Title:** Defining and Overcoming Hypoxic Immune Dysfunction in Head and Neck Cancer
**Time Commitments:** .12 calendar
**Supporting Agency:** V Foundation (Ishizuka)
**Address:** 14600 Weston Parkway Cary, NC 27513
**Contracting/Grants Officer:** Sommer Axner
**Performance Period:** 11/01/2021-11/01/2023
**Level of Funding**:
**Project Goals:** Identify mechanisms and therapeutic interventions to overcome hypoxia-induced immune suppression in head and neck tumors
**Specific Aims:** 1) Dissect mechanisms of hypoxia-induced immune dysfunction in patient tumor-immune co-cultures; 2) Delineate the effects of targeting ADAR1 in hypoxic head and neck tumors
**Overlap:** No scientific or budgetary overlap

**Title:** Yale Comprehensive Cancer Center Support Grant (CCSG); Developmental Therapeutics Program
**Time Commitments:** 1.2 calendar
**Supporting Agency:** NIH/NCI 5P30CA016359-41 (Winer)
**Address:**
9609 Medical Center Drive
MSC 9739 Bethesda, MD 20892-9739
**Contracting/Grants Officer:** Min He
**Performance Period:** 07/01/1997-07/31/2023
**Level of Funding**:
**Project Goals:** The major goal of the interactive Scientific Programs and Shared Resources within this project scope is to improve the understanding and treatment of human cancer.
**Specific Aims:** The Developmental Therapeutics Committee seeks to leverage exceptional structural and chemical biology and pharmacology research with early phase and disease-focused clinical research teams, to advance new treatments for cancer, including those aimed at hitherto undruggable targets, and in integration with immunotherapeutics**.** The Protocol Review Committee performs scientific review of new interventional human subjects research that is cancer related, and assesses priority within the cancer center.
**Overlap:** No scientific or budgetary overlap

**Title:** ECOG-ACRIN Operations Center- Clinical Trials
**Time Commitments:** 0.6 calendar
**Supporting Agency:** NCI U10 CA180820 Comis/Schnall (PI)
**Address:** 37 Convent Dr. Bethesda, MD 20814
**Contracting/Grants Officer:** Donna Marinucci
**Performance Period:** 03/01/2019-02/28/2024
**Level of Funding**:
**Project Goals:** The goal of this committee to improve survival and functional outcomes for patients with all stages of head and neck cancer.
**Specific Aims:** Favorable prognosis patients are studied in trials of treatment deintensification, while novel

targets are explored in patients with poor prognosis or recurrent disease.
**Overlap:** No scientific or budgetary overlap

**Title:** Translation and validation of non-invasive measurement of PD-L1 levels with positron emission tomography (PET) in head and neck malignancies and intracranial metastases
**Time Commitments:** 0.12 calendar
**Supporting Agency:** NCI/NIH (R21CA259964) Aboian (PI)
**Address:** National Cancer Institute 9000 Rockville Pike
Bethesda, MD 20824
**Contracting/Grants Officer:** Justin Birken
**Performance Period:** 12/01/2021-11/30/2023
**Level of Funding**:
**Project Goals:** Successful completion of this project will demonstrate that PET imaging of PD-L1 expression in primary and metastatic disease using an 18F-labeled adnectin tracer is feasible, correlates with PD-L1 expression levels on FDA approved IHC measurement from targeted biopsy, and can serve as an imaging biomarker of PD-L1 level in primary tumors and metastases in humans.
**Specific Aims:** Specific Aim 1: To develop and validate quantitative measurement of PD-L1 using PET. PD-L1 is a biomarker of tumor response to ICI therapy, but IHC measurement of PD-L1 in tumor metastases is invasive and is not always clinically feasible. Hypothesis: There is a correlation between quantitative measurement of PD-L1 levels with PET and IHC. In this aim, we will validate a novel PET tracer for measurement of PD-L1 levels in 12 patients with metastatic oropharyngeal squamous cell carcinoma undergoing ICI therapy and will correlate this measurement to established gold standard IHC measurements and time to recurrence after surgery. Specific Aim 2: To translate non-invasive measurement of PD-L1 with PET to intracranial metastatic lesions. Adnectin 18F-BMS-986192 PET tracer use has been established in lung cancer, but feasibility of using this tracer in brain metastases has not been established. Hypothesis: PD-L1 levels can be measured in the brain metastases from non-CNS primary cancer using quantitative PET and it correlates to IHC. In this aim, we will validate the adnectin PET tracer in measurement of PD-L1 in contrast enhancing intracranial metastases in 12 patients undergoing surgical resection and will determine the imaging parameters that allow accurate measurement of PD-L1 with respect to gold standard IHC.
**Overlap**: None

**Title:** Yale Comprehensive Cancer Center Support Grant (CCSG) **a multi-project award*
**Time Commitments:** 1.20 calendar
**Grant Component(s):** Leadership Planning and Evaluation
**Project Goals:** The major goal of the interactive Scientific Programs and Shared Resources within this project scope is composed to improving the understanding and treatment of human cancer.
Status of Support: Active
**Project Number:** 5P30CA016359-43
**Name of PD/PI:** Winer, Eric
**Source of Support**: NIH/NCI
**Primary Place of Performance**: Yale University.
**Performance Period:** (MM/YYYY) (if available): 07/01/1997 – 07/31/2024.
**Total Award Amount of Grant Component(s)** (including Indirect Costs):

**Title:** University of Pittsburgh Medical Center Head and Neck Cancer SPORE Project 2 title: Optimizing patient selection and deintensified therapy for HPV+ oropharynx cancer (OPC)
**Supporting Agency** can add P50CA097190
**Time Commitments:** .54
**Grants officer:** Dawn Walker
**Performance Period**: 09/14/2022- 08/31/2027
**Level of Funding:**

**Project Goals:** to refine patient selection criteria for primary surgical deintensification therapy of locoregionally confined HPV-associated oropharynx cancer by developing and validating a novel genomic and transcriptomic signature of high-risk neck disease in the completed ECOG 3311 trial of HPV+ OPC. As well, to determine the innate or adaptive immune pathways in high-risk patients, and whether PD1 immunotherapy plus 50 Gy RT provides promising oncologic and toxicity outcomes in such high-risk oropharynx cancer patients.

**Specific Aims: Aim 1. Develop and validate a novel radiogenomic signature of high-risk neck disease (HRND).** We will further refine a 4-gene signature of HRND using specimens from 200 additional UPMC tumors (100 Arm D-like specimens and 100 tumors similar to Arms A+B+C) (**UPMC OPC cohort**). We will then examine the ability of our radiomics approach, both alone and in combination with our mutational signature, to predict HRND in CT scans from the **UPMC OPC cohort**. These signatures will be evaluated alone or in combination, and then validated using the remaining available ECOG 3311 specimens (n=260, **ECOG 3311 cohort,** see letter).

**Aim 2. Examine the relationship between tumor mutations, immune infiltrate, and IFN signature in HRND.**
We will determine if clinically significant mutations in HPV + OPC are driving IFN-signaling and immune infiltrate. To that end, we will knock out each gene from our final signature, plus 2 additional genes (TRAF3 and CYLD) potentially associated with *increased* immune infiltrate, previously identified by our co-I Dr. Barbara Burtness (Hajek et al. Cancer 2017), in syngeneic OPC models, to determine effects on immune infiltrate and tumor growth at baseline or after RT + PD-1 blockade. Flow cytometry, RNAseq and IHC will analyze effects on immune infiltrate and downstream IFN-associated pathways following knockdown and PD-1 treatment.

**Aim 3. Evaluate the interaction between tumor mutation, immune infiltrate, peripheral blood IFN signature and outcome in OPC patients treated with TORS and adjuvant immunoRT.** This aim will test a new therapeutic deintensification approach for HRND patients. HCC 18-034 (NCT03715946) enrolls HPV+ OPC patients with HRND (eg. ECOG 3311 Arm D) surgically resected with TORS, followed by 50 Gy RT plus nivolumab. We will perform tumor WES and RNAseq, as well as cytokine analysis of peripheral blood, to examine the interaction between mutation, immune infiltrate, peripheral blood IFN signature and outcome.

**Overlap:** No scientific or budgetary overlap

**Pending**

**Title:** Combined Demethylation and Immune Checkpoint Inhibition in HPV-Associated Head and Neck Cancer
**Time Commitments:** 1.2 calendar
**Supporting Agency:** NCI/NHI Burtness (PI)
**Address:** 37 Convent Dr. Bethesda, MD 20814
**Contracting/Grants Officer:** Pending
**Performance Period:** 09/01/2021-08/31/2026
**Level of Funding**:
**Project Goals:** The goal of this project is to determine whether demethylation therapy primes for immune checkpoint inhibition in HPV-associated oropharynx cancer, quantitating anti-tumor response and immune

activation with radiographic, histologic, immunoprobing and measurement of cancer-specific neoantigens in a 3-arm clinical trial comparing 5-azacytidine, nivolumab or the combination in the pre-operative setting. As well, 18FAra-G metabolic imaging for non-invasive measurement and spatial characterization pre- and post-treatment will be performed.

**Specific Aims:** Specific Aim 1: **Determine the combined effect of demethylation and PD-1 axis blockade in a clinical trial in HPV+ HNSCC.** As reversal of hypermethylation in HPV-associated cancers undoes immunosuppressive silencing of immune response genes, permitting infiltration of activated T cells and priming for ICI, **we hypothesize that combination 5-azaC and nivolumab will significantly enhance immune and clinical response relative to either single agent.** We will conduct a randomized 3-arm window trial of 5-azaC, nivolumab or the combination, prior to resection of HPV+ HNSCC. The primary trial endpoint will be anticancer efficacy based on the pathologic score of immune response, with a secondary endpoint of T cell infiltration.

Specific Aim 2. **Non-invasively quantify tumoral infiltration by activated T-cells by $^{18}$F-AraG PET imaging after 5-azaC, nivolumab or combination therapy.** We anticipate that T cell activation due to combination 5-azaC and nivolumab will be reflected in increased tumoral uptake of $^{18}$F-AraG relative to pre-treatment PET imaging. Patients will undergo $^{18}$F-AraG PET imaging before and after 1 cycle of 5-azaC, nivolumab or combination therapy. Change in PET uptake derived from kinetic modeling approaches will be correlated with measures of T cell infiltration and activation, with evidence of antitumor effect reflected in cell death as assayed in Aim 1, and with tumor size on baseline imaging. **We hypothesize that we will be able to detect augmented activated T cell infiltration following each of the therapies, but that the magnitude of activated T cell infiltration and anticancer effect will be greatest in the combination arm.** The success of this aim will validate the use of $^{18}$F-AraG as a PET imaging biomarker to monitor treatment with strategies influencing activated T cell infiltration, potentially guiding the duration or intensity of such therapy in the future.

Specific Aim 3: **Evaluate measures of T-cell clonality and activation following demethylation.** Our preliminary data show that demethylation of HPV+ HNSCC is associated with expression of cancer-specific antigens including Line-1 ORF2, and 4 GAGE proteins. From a prior window trial of HPV+ HNSCC patients, we noted demethylation increased T cell infiltration into the tumor. **We hypothesize that demethylation therapy will cause emergence of T cell clones that are activated by peptides from newly expressed neoantigens in tumor cells, and that the combination of demethylation and PD-1 therapy will further enhance T cell activation**. We will test post-treatment tissue and blood to determine if T cells expand in response to peptides from these cancer-specific antigens and compare response in the three arms of the trial. Such neoantigens could themselves constitute future therapeutic targets.

**Overlap**: None

**Previous**

**Title:** STING Biomarker and Therapeutic Biomarkers in HNSCC
**Time Commitments:** 0.12 calendar
**Supporting Agency:** Yale Cancer Center **Address:**
333 Cedar Street, New Haven, CT 06520
**Contracting/Grants Officer:** Christine Holmberg
**Performance Period:** 02/01/2018-01/31/2023
**Level of Funding**:
**Project Goals:** The goals of this project are to evaluate the prognostic value of STING expression in HNSCC TMA cohorts with defined treatment regimens; to identify tumor expression or mutation profiles associated with STING loss through RNA and DNA sequencing; and to initiate a phase I HNSCC trial combining STING

agonists with radiation therapy and/or cisplatin chemotherapy in patients with tumor or stromal STING expression detected by IHC.
**Specific Aims:** *Aim 1* is to evaluate the prognostic value of STING expression in HNSCC TMA cohorts with defined treatment regimens. Tumor and stromal staining will be scored in the context of 1) other HNSCC tumor biomarkers and 2) immune system markers in order to determine the relationships between STING expression, immune surveillance, therapeutic response, and patient outcomes.
*Aim 2* is to identify tumor expression or mutation profiles associated with STING loss through RNA and DNA sequencing. These profiles may identify molecular mechanisms for loss of STING expression and suggest dependent pathways that could be targeted in this patient subset.
*Aim 3* is to initiate a phase I HNSCC trial combining STING agonists with radiation therapy and/or cisplatin chemotherapy in patients with tumor or stromal STING expression detected by IHC.
**Overlap:** No scientific or budgetary overlap

**Title:** Novel Therapies and Targets in Head and Neck Cancer
**Time Commitments:** 0 calendar
**Supporting Agency:** Yale Comprehensive Cancer Center Yale Translational-Targeted Area of Research Excellence (T-TARE) Grant Head and Neck Cancer Program
**Address:** 333 Cedar Street WWW225 New Haven, CT 06510
**Contracting/Grants Officer:** None
**Performance Period:** 07/01/2016-06/30/2017
**Level of Funding**:
**Project Goals:** To develop pilot data supporting a SPORE application in head and neck cancer.
**Specific Aims:** Aim 1: To determine the mechanism of cell death and synergy between Wee1 and Aurora A inhibition, whether this is cell cycle dependent, and what the role of additional checkpoint kinases and DNA damage response genes are in sensitivity/resistance to the novel combination.
Aim 2: To confirm the synergy of dual Wee1 and Aurora A inhibition at relevant doses in xenograft models, test candidate pharmacodynamic surrogate biomarkers emerging from the mechanistic studies conducted in Aim 1.
Aim 3: To evaluate whether the response of the PD-1 inhibitor pembrolizumab in patients with persistent disease will increase following completion of chemoradiation for locally advanced HNC.
Aim 4: To examine whether radiation induced DNA damage will amplify the repertoire of mutations and of potential novel antigens.
Aim 5: To investigate APOBEC3B expression level and identify possible interacting partners in relation to viral infection and tobacco exposure in HNC and to investigate the molecular partnership and mechanism that distinguish the impact of APOBEC3B in viral response and in tobacco associated cancer.
Aim 6: To extend preliminary findings that treatment nucleoside analogs can upregulated APOBEC3B expression and validate this is pre- and post-treatment specimens from an on-going window trial.
**Overlap**: None

**Title:** Epigenetic Mechanisms of Inflammation and Fatigue in Head and Neck Cancer Patients
**Time Commitments:** .6 calendar
**Supporting Agency:** NINR/NIH/DHHS 5R01NR015783-03
**Address:** 6701 Democracy Blvd., Room 710, One Democracy Plaza, Bethesda, MD 20892-4870
**Contracting/Grants Officer:** Brian Albertini
**Performance Period:** 04/01/2018-03/31/2019
**Level of Funding**:
**Project Goals:** The goal of the proposed research (R01) is to investigate epigenetic mechanisms of inflammation and fatigue in patients with head and neck cancer (HNC) undergoing chemotherapy plus intensity-modulated radiotherapy (chemoIMRT).
**Specific Aims:** Specifically, we are examining 1) whether DNA methylation changes are associated with

inflammation and fatigue during the acute phase of chemoIMRT and 2) whether acute DNA methylation changes persist following chemoIMRT and remain associated with inflammation and fatigue.
**Overlap**: None

**Title:** Yale SPORE in Lung Cancer (YSILC): The Biology and Personalized Treatment of Lung Cancer Developmental Research Program Award: Combined AURKA and WEE1 targeting inhibits a druggable effector pathway in *KRAS*-mutant lung adenocarcinoma
**Time Commitments:** 0 calendar
**Supporting Agency:** NIH/NCI 2P50CA196530-06 (DRP)
**Address:** 9609 Medical Center Drive, Room 3W106 MSC
9726 Bethesda, MD 20892
**Contracting/Grants Officer:** Peter Ujhazy
**Performance Period:** 08/01/2020-07/31/2022
**Level of Funding**:
**Project Goals:** The goal of this project is to test the efficacy of combined AURKA and WEE1 inhibition in *KRAS*-mutant lung adenocarcinoma models, as well as to explore whether AURKA inhibition or the combination of an AURKA inhibitor with a WEE1 inhibitor can prevent AURKA-mediated adaptive resistance to direct $KRAS^{G12C}$ inhibitors.
**Specific Aims:** Specific aim 1 is to determine the effects of combined AURKA and WEE1 inhibition in *KRAS* mutant and wild type cell lines, and in cells with adaptive resistance to KRAS inhibition, using mechanistic insights to propose predictive biomarkers.
Specific Aim 2. Confirm synergy in xenograft models of KRAS mutant and wild-type NSCLC, with assessment of the utility of biomarkers evaluated in Aim 1.
**Overlap**: None