AWARD NUMBER: W81XWH-22-1-0722

TITLE: Treating Oral Cancer Pain with LRP1 Agonists

PRINCIPAL INVESTIGATOR: Wendy Campana, PhD

CONTRACTING ORGANIZATION: University of California, San Diego
La Jolla, CA

REPORT DATE: October 2023

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2023	Annual	30Sep2022-29Sep2023

4. TITLE AND SUBTITLE

Treating Oral Cancer Pain with LRP1 Agonists

5a. CONTRACT NUMBER
W81XWH-22-1-0723

5b. GRANT NUMBER
CP210088

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER
202698

5e. TASK NUMBER
1

5f. WORK UNIT NUMBER

6. AUTHOR(S)

Wendy M Campana, PhD (contact PI)
Yi Ye, PhD

E-Mail: wcampana@health.ucsd.edu; yy22nyu@nyu.edu

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

University of California, San Diego
9500 Gilman Drive
La Jolla, CA 92037

NEW YORK UNIVERSITY
70 Washington Square S
New York, NY 10010

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Oral cancer pain is frequently accompanied by other psychosocial comorbidities such as anxiety, depression, fatigue, and sleep disorders. Unfortunately, existing treatments for chronic pain (*i.e.*, opioids) are ineffective and associated with deleterious side effects, greatly worsening what is already the substantial burden of cancer. Our research program aims to elucidate the basic causes of chronic oral cancer pain, and to identify new treatments for chronic pain. We recently discovered a novel cell receptor system that has cell survival and anti-inflammatory properties. Activators of the receptor system reduced acute pain and prevented the development of chronic pain in our preclinical models. Our preliminary studies suggest that the mechanism of action includes regulating activities of specialized glia and inflammatory cells in the peripheral nerve and keeping pain transmitting neurons healthy. Herein, we will test whether our receptor activators function in combinate with specialized glia cells to antagonize inflammation and prevent oral cancer pain. Studies planning to test effects on pain behaviors and cellular and molecular mechanisms are underway. We also plan to identify novel populations of cells in tumor tissues that contribute to oral cancer pain. A consideration of great importance in this work is whether novel drugs that activate this receptor system can improve chronic pain such as oral cancer pain. This information is essential for human clinical trials, and is consistent with our overarching research vision to understand the causes and develop effective treatments for chronic pain.

15. SUBJECT TERMS None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION

Oral cancer is very common (4 times more than the civilian) in our active military and veteran populations. Pain is rated as the worst symptom, and patients have difficulty in eating, drinking, and talking. Oral cancer pain is frequently accompanied by other psychosocial comorbidities such as anxiety, depression, fatigue, and sleep disorders. Unfortunately, existing treatments for chronic pain (*i.e*., opioids) are ineffective and associated with deleterious side effects, greatly worsening what is already the substantial burden of cancer. Our research program aims to understand the basic causes of chronic oral cancer pain, and to identify new treatments for chronic pain. We recently discovered that low-density lipoprotein receptor related protein (LRP1) has cell survival and anti-inflammatory properties. Activators of the receptor system reduced acute pain and prevented the development of chronic pain in our preclinical models. The mechanism of action includes regulating activities of Schwann cells and macrophages that suppresses neuroinflammation and neuronal excitability. We hypothesize that LRP1 agonists produce pain relief in part by immune and glial modulation that control neuroinflammation in oral cancer. We propose a systematic set of studies to first identify the behavioral and electrophysiological mechanisms associated with pain outcomes in models of oral cancer, and how these outcomes are regulated by LRP1 activation or SC LRP1 deletion (**Aim 1**). We then examine the molecular and cellular mechanisms underlying SC and immune interactions that contribute to inflammation and tumor progression using flow cytometry and single-cell RNA sequencing (**Aim 2**).

2. KEYWORDS

LRP1, oral cancer, pain, neuroinflammation, macrophages

3. ACCOMPLISHMENTS:

In this progress report we have accomplished studies associated with Major task 1, Major task 2 and Major task 4. We highlight these in our accomplishments below.

Major Task 1: Perform orthotopic, syngeneic cancer cell inoculations with and without LRP1 agonists in wild type male and female mice and measure pain related behaviors (UCSD and NYU).

1) We have made substantial progress in optimizing and determining baseline measurements for our oral cancer pain model. First, we cultured the MOC1 cell line, an oral cancer cell line that has less aggressive growth, that will be used to induce oral cancer in our mouse model. We determined the doubling time of MOC1 *in vitro* and will apply this growth rate parameter to our studies in vivo. This will help us control the actual tumor size *in vivo*.

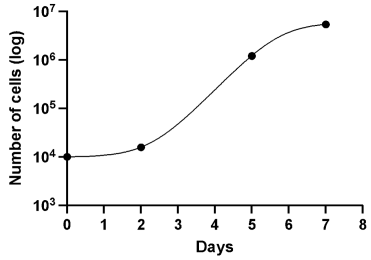


Fig. 1. We determined the doubling time (12h) of MOC1 cells

2) We have measured pain related behaviors in both the scLRP1$^{+/+}$ mice and the scLRP1$^{-/-}$ mice. These mice will be used for our oral cancer pain model, and thus determining their baseline pain-related behaviors prior to inducing oral tumor formation will be helpful for accurate interpretation of the post tumor pain related behavior studies. We determined that scLRP1$^{-/-}$ mice have increased facial nociception scores ($p<0.05$), decreased facial thermal latencies ($p<0.001$) and reduced time in the inner zone test ($p<0.05$). Further, we demonstrate that baseline pain responses are different when considering sex. Male mice have greater nociceptive scores compared to females, while female mice have spent shorter times in the inner zone as compared to male mice. These findings suggest that we need to consider sex when planning our oral cancer pain studies.

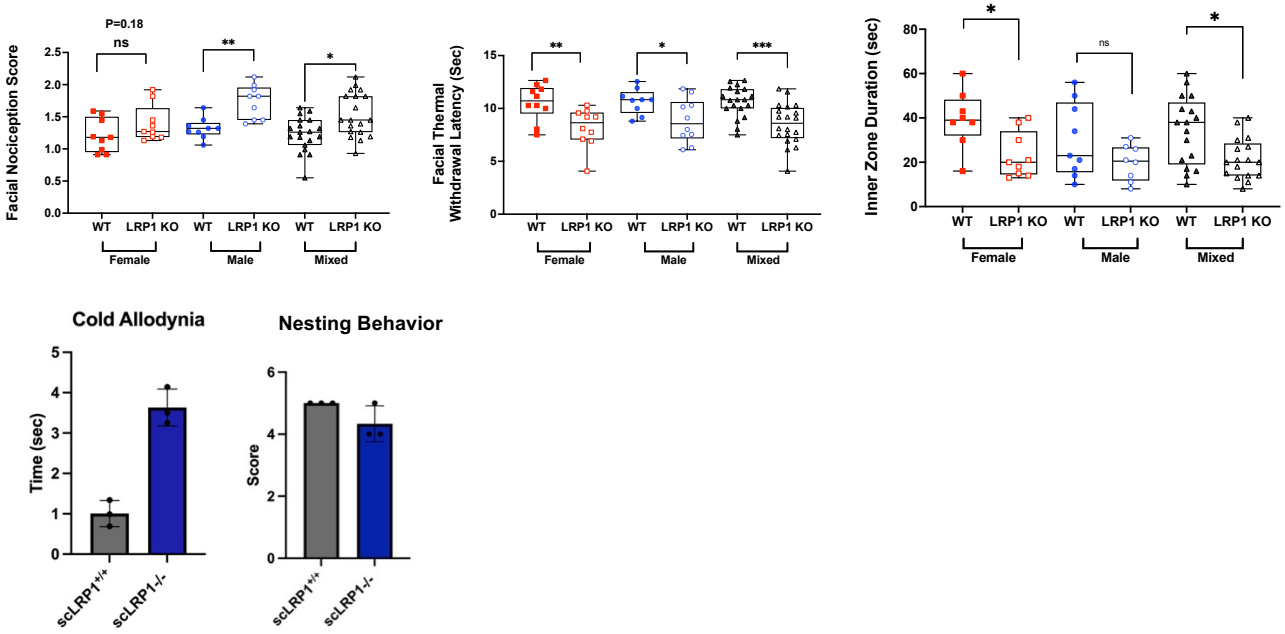


Fig. 2. scLRP1$^{-/-}$ mice exhibited increased facial mechanical allodynia (more obvious in males), thermal hypersensitivity, and spent less time in the inner zone (significant in females), compared to the scLRP1+/+ mice littermate controls. scLRP1-/- mice also demonstrated increased cold allodynia at baseline and a slight decrease in nesting behaviors, compared to scLRP1$^{+/+}$ mice.

3) We performed a pilot study using the two-bottle assay to examine capsaicin sensitivity in female wild type and scLRP1$^{-/-}$ mice. We noted a huge variation in the two-bottle assay. We are in the process of optimizing the assay. Alternatively, we can use a new OPAD system that we are currently optimizing for licking, mechanical, and thermal sensitivities in the orofacial region.

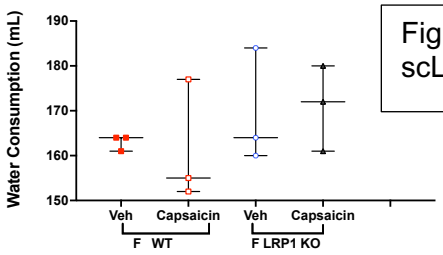


Fig. 3. The two-bottle capsaicin assay in scLRP1$^{+/+}$ and scLRP1$^{-/-}$ mice (n=3/group).

4) We have performed initial studies with the MOC2 cell line. The MOC2 cell line is a more aggressive cell line and is used to develop oral cancer tumor. Male scLRP1$^{-/-}$ mice exhibited increased facial allodynia when inoculated with MOC2 cells into the tongue. These data demonstrate a trend in nociceptive changes but will require additional mice to reach statistical significance.

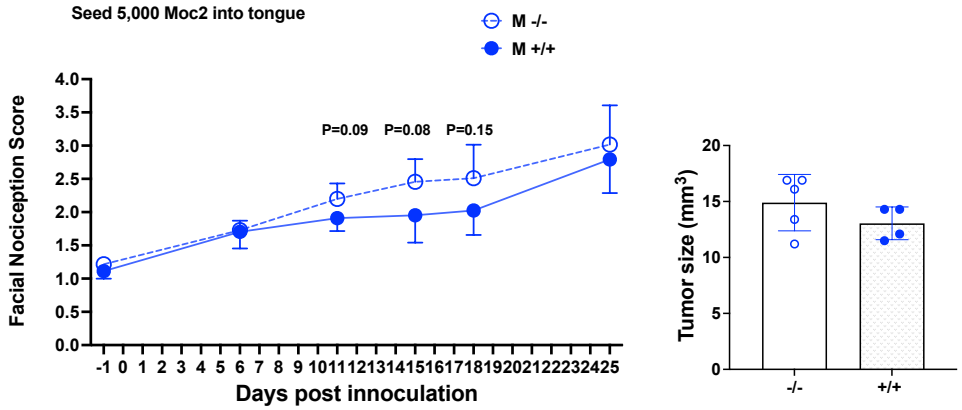


Fig. 4. 5000 MOC2 (mouse oral cancer) cells were inoculated into the tongue of scLRP1$^{-/-}$ (n=5) and scLRP1$^{+/+}$ (n=4) male mice. Mice were followed for 25 days.

Major Task 2: Evaluate neuronal sensitization in mouse models of oral cancer using a time window when LRP1 agonists exert a significant reduction in pain-like behaviors (NYU)

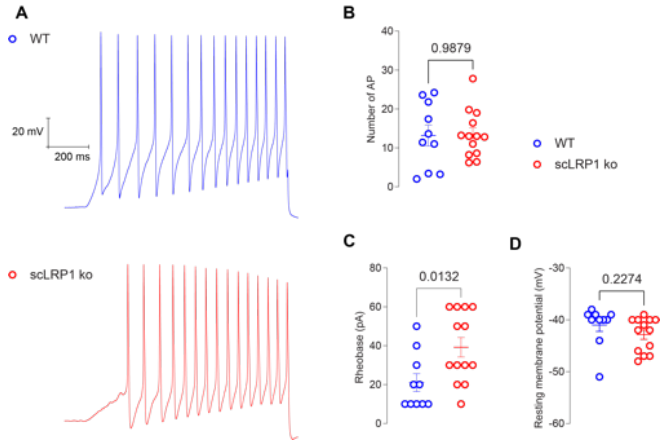


Fig. 5. Neuronal excitability was determined by firing frequencies, rheobase, and resting membrane potentials.

5) We are working on whole-cell patch recordings in TG neuronal cultures isolated from scLRP1$^{-/-}$ and scLRP1$^{+/+}$ mice. We note differences in the rheobase; scLRP1$^{-/-}$ mice have increased rheobase.

Major Task 4: Perform single-cell RNASeq (scRNASeq) in the trigeminal ganglia (TG) of mice with oral cancer (UCSD and NYU)

6) Because the subtle sex-difference we observed in behavior, we performed bulk RNAseq in mouse TGs from female and male scLRP1$^{-/-}$, scLRP1$^{+/+}$ mice to screen for genes that can explain for sex-difference. Female and male scLRP1$^{-/-}$ exhibit distinct gene expression profiles and pathways. These studies will help shape the experimental design for the single cell RNA Seq studies in the TG during oral cancer pain.

Female TG

SYMBOL	LFC	Padj
Lamp5	3.70	0.01
Fam135b	3.05	0.00
Slc5a7	2.85	0.01
Slc12a5	2.39	0.00
Gad2	2.22	0.00
Grin2b	2.10	0.00
A330076C08I	2.06	0.00
Rmst	2.05	0.00
Gm3764	2.03	0.00
Pcdhga1	-1.54	0.00
Bpifb1	-1.65	0.04
Gucy2f	-1.88	0.00

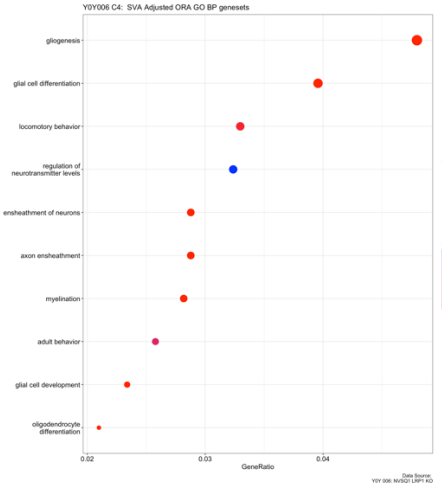
Male TG

SYMBOL	LFC	Padj
4933413L06I	1.37	0.00
Chil3	1.05	0.00
Cxcl10	-1.24	0.00
Gm14966	-1.33	0.00
Xlr4b	-1.38	0.00
Retnla	-1.46	0.00
Inmt	-1.50	0.00
Sall1	-1.53	0.00
Kcne4	-1.59	0.00
Bglap	-1.65	0.00
Tdgf1	-1.66	0.00
Opalin	-1.79	0.00

Fig. 6. DEGs between female scLRP1$^{-/-}$ vs. scLRP1$^{+/+}$ mice are different from DEGs between male scLRP1$^{-/-}$ vs. scLRP1$^{+/+}$. Table on the left shown top 10 upregulated or downregulated genes using logfoldchange (LFC)=1, Padj<0.05.

7) Pathway analysis in mouse TGs from female and male scLRP1$^{-/-}$, scLRP1$^{+/+}$ mice. We are in the process of analyzing and sorting these datasets.

Female TG GO BP



Female TG KEGG

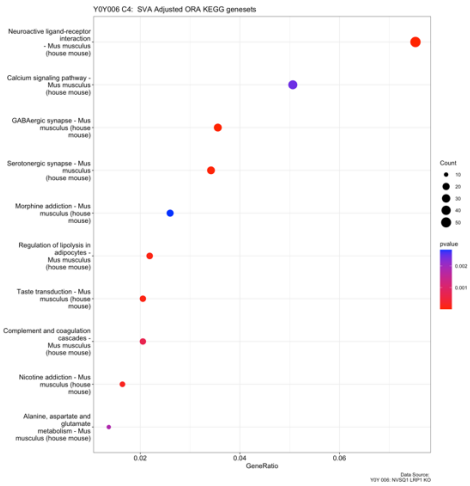
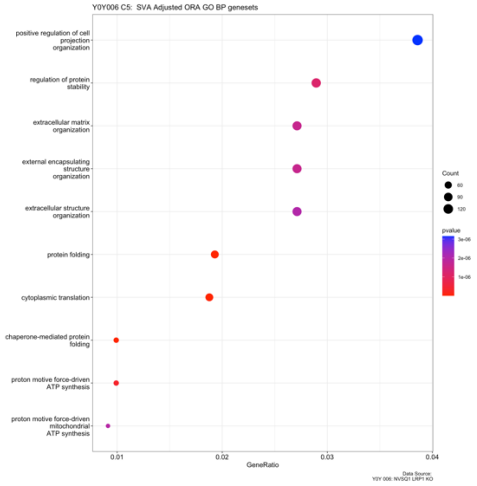
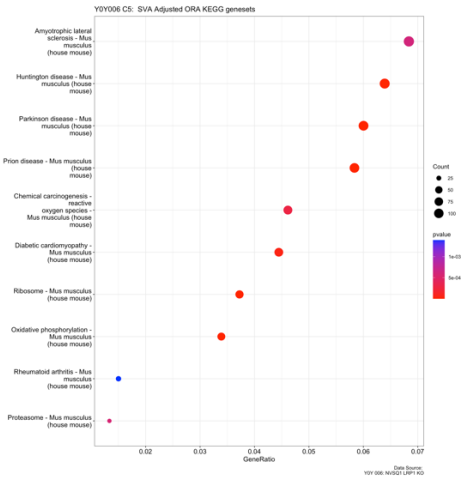


Fig. 7. Top 10 most altered biological pathways (BP) and KEGG are distinct between between female scLRP1$^{-/-}$ vs. scLRP1$^{-/-}$ mice, using scLRP1$^{+/+}$ mice with the same sex as controls.

Male TG GO BP



Male TG KEGG



Goals of Project. We hypothesize that LRP1 agonists produce pain relief in part by immune and glial modulation that control neuroinflammation in the tumor environment during oral cancer pain.A critical component of successful translation is the determination of LRP1 activation on pain outcomes. Our proposal will expand our understanding of cancer pain biology in clinically relevant models of oral cancer pain and the impact of innovative treatments on functional sensory recovery. We aim to achieve two key objectives:

Aim 1 will compare the electrophysiological and behavioral effects of LRP1 agonists, EI-tPA and SP16, and targeted deletion of SC LRP1 on oral cancer pain

Aim 2 will examine the effects of EI-tPA and SP16, and targeted deletion of SC LRP1 on inflammation and tumor progression

Abbreviations: LRP1- low-density lipoprotein receptor related protein (LRP1), SC - Schwann cell, EI-tPA - enzymatically inactive - Tissue-type plasminogen activator; MOC- mouse oral cancer, scRNAseq - single cell RNA sequencing

Research Progress

Dissemination of Results

One manuscript is currently under preparation.

Plans for Next Reporting Period

Priorities for the next reporting period include:

1. Optimize dosing paradigm of LRP1 agonists for pain treatment and measure behaviors in mice inoculated with either MOC1 or MOC2 cells in WT, scLRP1$^{+/+}$, and scLRP1$^{-/-}$ mice.
2. Complete electrophysiological recordings in WT and scLRP1$^{-/-}$ mice.
3. Measure cytokine expression by mouse cytokine arrays in tongues of C57BL/6 mice groups generated in Subtask3
4. Optimize protocols and Isolate RNA from tongues for single cell RNA Seq

4. IMPACT

Impact on principal discipline.

Nothing to Report

Impact on other disciplines.

Nothing to Report

Impact on technology transfer.

Nothing to Report

Impact on society.
Nothing to Report

5. 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.

Both the contact PI (Campana) and the partnering PI (Ye)'s lab experienced personnel loss due to Covid and had difficulty recruiting new lab members. Dr. Ye has successfully recruited two new postdoctoral fellows that are expected to start in April 2024. Dr. Campana has successfully recruit a new Research Associate that started in late August 2023.

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

6. PRODUCTS

Nothing to Report

* Papers included in appendix

Journal publications

Books

Other

Websites

Technologies and techniques.

Inventions and patents.

Other products.

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS:
Individuals who have worked on project

University of California, San Diego

Name:	Wendy M Campana, PhD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-1075-5584
Nearest person month worked:	3.0 calendar

Contribution to Project:	Project supervision, experiment design, and research administration
Funding Support:	W81XWH-22-1-0723; NIH R43CA268700 01; VA I01RX003363-04

Name:	Stefano Martellucci
Project Role:	Post Doctoral Fellow
Researcher Identifier (e.g. ORCID ID):	0000-0002-3952-3162
Nearest person month worked:	3 calendar
Contribution to Project:	Molecular and cellular assays,
Funding Support:	W81XWH-22-1-0723; VA I01RX003363-04

Name:	Miles Vecchitto
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.0 calendar
Contribution to Project:	Mouse colony maintenance, tissue collection
Funding Support:	W81XWH-22-1-0723

Name:	Zixuan Wang
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	0000-0003-2037-2509
Nearest person month worked:	1.76 calendar
Contribution to Project:	Mouse colony maintenance, pain related behavioral testing
Funding Support:	W81XWH-22-1-0723; UCOPC23CR5549

NEW YORK UNIVERSITY

Name:	Yi Ye, PhD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-1075-5584
Nearest person month worked:	2.40 calendar
Contribution to Project:	Project supervision, experiment design, and research administration
Funding Support:	W81XWH-22-1-0723; 1R01DE032501-01; 5R01DE029493-03

Name:	Naijang Liu
Project Role:	Associate Research Scientist
Researcher Identifier (e.g. ORCID ID):	0000-0003-2738-3147
Nearest person month worked:	1.05 calendar
Contribution to Project:	Behavioral characterization
Funding Support:	W81XWH-22-1-0723; 1R01DE032501-01; 5R01DE029493-03

Name:	Gisella Campanelli
Project Role:	Post Doctoral Associate
Researcher Identifier (e.g. ORCID ID):	0000-0003-3946-0066
Nearest person month worked:	1.05 calendar
Contribution to Project:	Cell culture
Funding Support:	W81XWH-22-1-0723; 1R01DE032501-01; 5R01DE029493-03

Name:	Morgan Zhang

Project Role:	Associate Research Scientist
Researcher Identifier (e.g. ORCID ID):	0000-0001-7670-5304
Nearest person month worked:	0.75 calendar
Contribution to Project:	Mouse colony maintenance, tumor inoculation, behavioral modeling
Funding Support:	W81XWH-22-1-0723; 1R01DE032501-01; 5R01DE029493-03

Other organizations.

We received pharmaceutical grade SP16 for our pain studies in oral cancer from Serpin Pharma

8. SPECIAL REPORTING REQUIREMENTS

This is a collaborative award between Yi Ye (New York University) and Dr. Wendy Campana (University of California, San Diego). Over the last year, Dr. Yi and Campana have met jointly twice per month by zoom to discuss experimental plans and results. In addition, we are drafting a manuscript together based on our initial results this year.

9. APPENDICES

Nothing to report