AWARD NUMBER: W81XWH-22-1-0501

TITLE: Understanding and Harnessing Therapy-Induced Senescence to Treat Ovarian Cancer

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CONTRACTING ORGANIZATION: University of Melbourne

REPORT DATE: JULY 2023

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2023	Annual	1 July 2022 – 30 June 2023

4. TITLE AND SUBTITLE
Understanding and Harnessing Therapy-Induced Senescence to Treat Ovarian Cancer

5a. CONTRACT NUMBER
W81XWH-22-1-0501

5b. GRANT NUMBER
OC210056

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
0011752125-0001

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
UNIVERSITY OF MELBOURNE
THE UNIVERSITY OF MELBOURNE
GRATTAN ST
PARKVILLE 3010

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
Conventional therapies for high-grade serous ovarian cancer (HGSC) often fail to eradicate tumors. The residual cancer cells that survive treatment can enter a state known as therapy-induced senescence (TIS). Despite TIS being a state of cell cycle arrest, TIS can have potentially adverse effects underpinning the development of treatment-resistant recurrent disease. Harnessing the properties of senescent cells can lead us to devise new therapeutic strategies that prevent recurrence, but little is known about TIS in HGSC. To better understand TIS in HGSC, we are using a suite of human and patient-derived cell lines and immune-competent model systems. We have performed gene expression and proteomic profiling of TIS HGSC cells and identified candidate targets involved in their cell survival and escape. We have also performed genetic and drug screens to highlight potential strategies for both (i) re-instating cell cycle arrest in cells that have escaped TIS and (ii) eradicating senescent HGSC cells. The next phase of the project will involve validating these targets and identifying in vivo approaches to exploit TIS for HGSC therapy.

15. SUBJECT TERMS
High-grade serous ovarian cancer, therapy-induced senescence, senolytic

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

Background: Although DNA-damaging chemotherapy and radiotherapy have been the cornerstone of ovarian cancer treatment for the past several decades, they often fail to completely eradicate tumors. The cancer cells that persist, surviving despite treatment, ultimately threaten patients' lives. The hope is that cancer cells exposed to these therapies will respond by undergoing apoptosis (programmed cell death). However, in many cases they survive and enter a state known as therapy-induced senescence (TIS). Despite senescence being a state of cell cycle arrest, TIS can be disastrous, underpinning the development of recurrent, treatment-resistant disease because it (i) protects cancer cells from apoptosis, (ii) is "escapable," evidenced by resumption of proliferation in TIS cells over time and (iii) can be immunosuppressive via the senescence secretome (the senescence-associated secretory phenotype (SASP)). Classical cellular senescence is typically studied in benign primary cells, where it is characterized by a permanent cessation of proliferation due to robust activation of intact p53 or RB tumor suppressor pathways. In contrast, cancer cells have often developed means to bypass these intrinsic growth-suppressive programs, as exemplified by high-grade serous ovarian cancers (HGSCs) that universally harbor TP53 mutations and frequently show RB1 inactivation. Despite these alterations, HGSCs can still enter TIS; however, the mechanisms governing TIS biology and its adverse effects in HGSC remain poorly understood.

Research Goal: Understanding the mechanisms underlying TIS in HGSC will reveal new opportunities to target TIS cells, mitigate their adverse properties and identify transformative treatments for ovarian cancer that prevent disease recurrence.

Specific Aims:
Aim 1. **Define the TIS phenotype in HGSC.**
Aim 2. **Identify novel strategies to eradicate senescent HGSC cells (senolytics).**
Aim 3. **Identify approaches to re-instate TIS *in vivo*.**

Keywords

Therapy-induced senescence (TIS), high-grade serous ovarian cancer (HGSC), senolytic

Accomplishments

Specific Aim 1: Define the TIS phenotype in HGSC

Major Task 1: Obtain approvals for use of human tissue use and animal studies. **COMPLETE**

Subtask 1: Obtain HRPO approval for human tissue use (AOCS-14, AOCS-30 patient-derived cell lines; AOCS-20 ascites for derivation of organoids). **COMPLETE**

Subtask 2: Submission of institution approved animal protocols for DoD's ACURO approval. **COMPLETE**

Subtask 3: Receive ACURO approval before initiating animal experiments. **COMPLETE**

Major Task 2: Perform RNA sequencing of 3 established HR-proficient cell lines (OVCAR-3, OVCAR-4, OVCAR-8), 2 patient-derived lines (AOCS-14, AOCS-30), 2 murine immune-competent lines (ID8 *Trp53*$^{-/-}$*/Pten*$^{-/-}$, ID8 *Trp53*$^{-/-}$*/Nf1*$^{-/-}$). **COMPLETE**

Subtask 1: Analysis of differential gene expression between proliferating and therapy-induced senescent cells. **COMPLETE**

Subtask 2: Derivation of TIS gene signatures. **COMPLETE**

We have compared our gene expression data with public datasets and signatures associated with senescence phenotypes. We find that HGSC TIS gene expression signatures are consistent with those identified in a stress response cluster as reported by (Zhang et al. Sci Adv 2022), senescent, and persister cells.

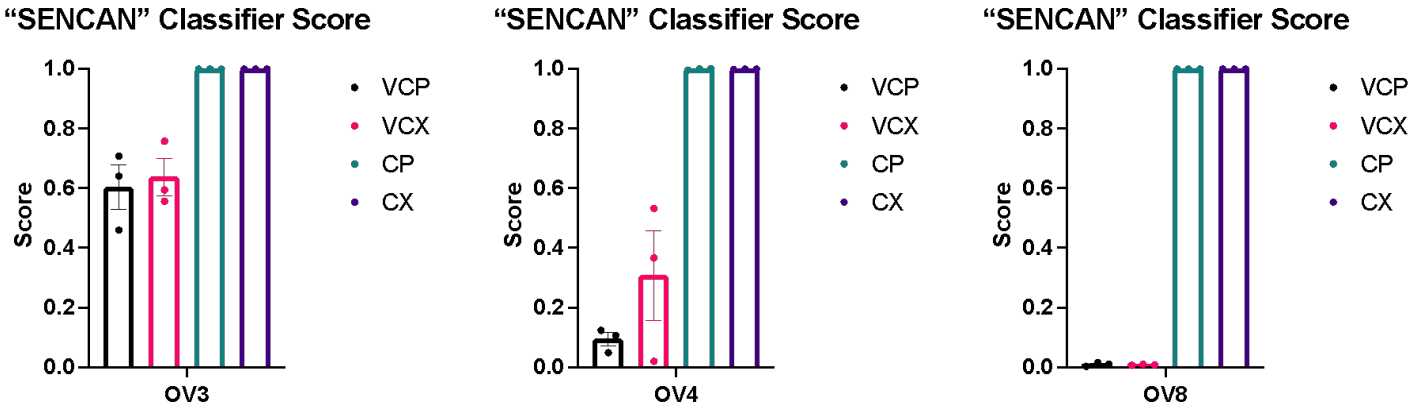

Figure 1. We analysed our RNAseq data OVCAR-3 (OV3), OVCAR-4 (OV4), OVCAR-8 (OV8) treated with vehicle (VCP, VCX), cisplatin (CP) or CX-5461) with respect to a SENCAN Classifier Score derived from transcriptomes of many human cancer cell lines treated with known chemotherapeutic drugs (Jochems et al. Cell Reports 2021).

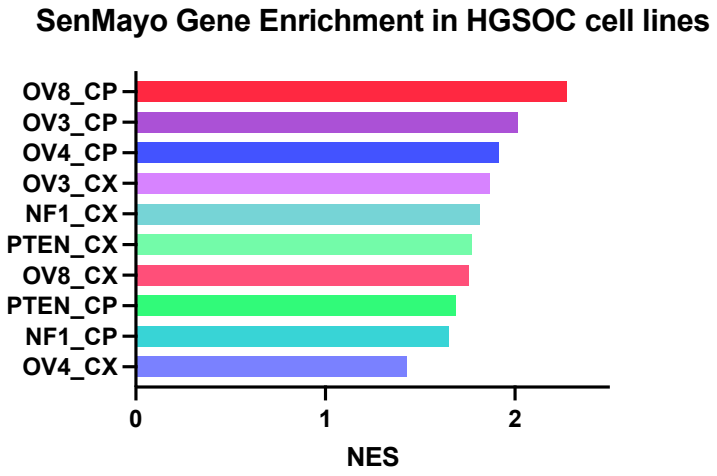

Figure 2. We compared our RNAseq dataset to SenMayo, a panel of 125 genes from various age-related datasets (Saul et al. Nature Communications 2022). PTEN, NF1 represent murine ID8 p53-/-/Pten-/- and p53-/-/Nf1-/- derivatives.

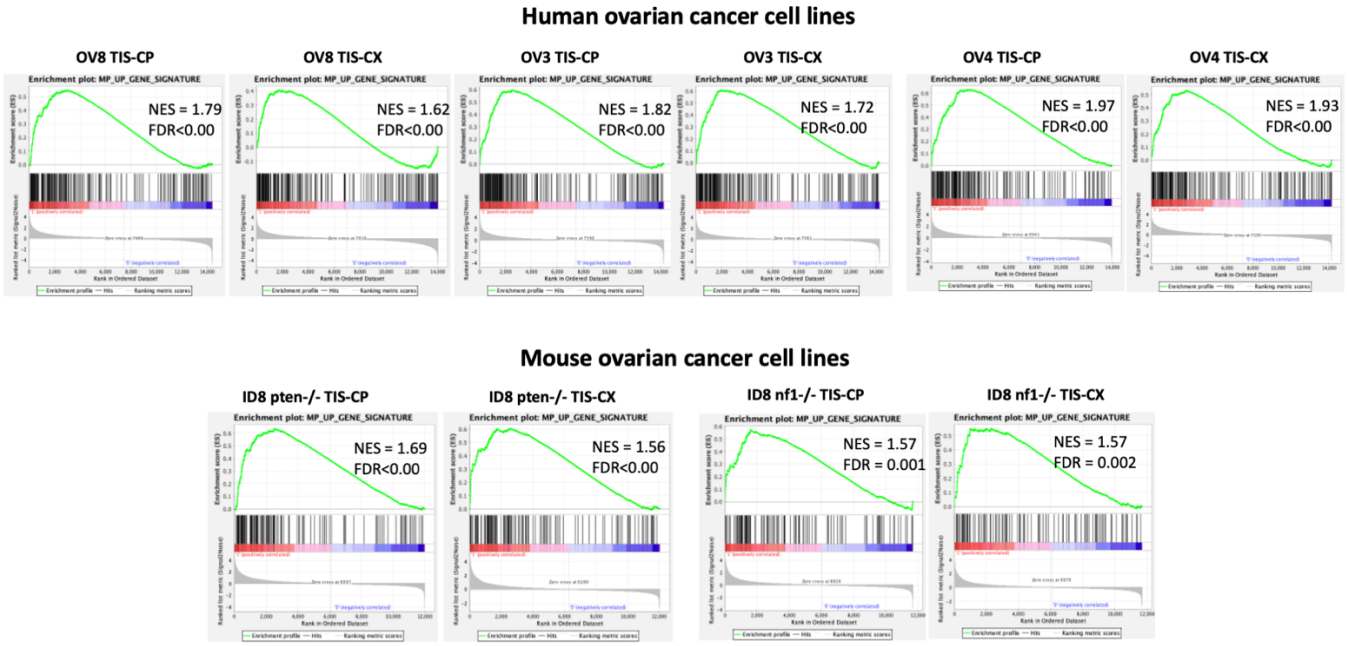

Figure 3. We found enrichment of a mTOR-dependent persister cell signature from our RNAseq data (Liu et al. Nature Communications 2022).

Major Task 3: Perform ATAC sequencing of 3 established HR-proficient cell lines (OVCAR-3, OVCAR-4, OVCAR-8), 2 patient-derived lines (AOCS-14, AOCS-30), 2 murine immune-competent lines (ID8 *Trp53*$^{-/-}$*/Pten*$^{-/-}$, ID8 *Trp53*$^{-/-}$*/Nf1*$^{-/-}$). **In progress**

Major Task 4: Validate RNA and ATAC sequencing data in AOCS-20 patient-derived organoids. **In progress**

Milestone #1: Establish key gene expression and chromatin accessibility profiles that can be potential targets of TIS for treating HGSC

We have identified key differentially expressed genes between proliferating and TIS HGSC cells. We rationalized that the differentially upregulated genes could be prioritized targets to be tested in the screens for Aim 2.

Major Task 5: Perform cell surface proteomics of 3 established HR-proficient cell lines (OVCAR-3, OVCAR-4, OVCAR-8), 2 patient-derived lines (AOCS-14, AOCS-30), 2 murine immune-competent lines (ID8 *Trp53*$^{-/-}$*/Pten*$^{-/-}$, ID8 *Trp53*$^{-/-}$*/Nf1*$^{-/-}$). **We have completed proteomics profiling of all cell lines indicated in Major Task 5.**

Subtask 1: Prepare samples for mass spectrometry of membrane and cell surface proteins

Subtask 2: Analysis of cell surface protein patterns on therapy-induced senescent and proliferating cells

We broadened our scope to include both cell surface and intracellular proteins.

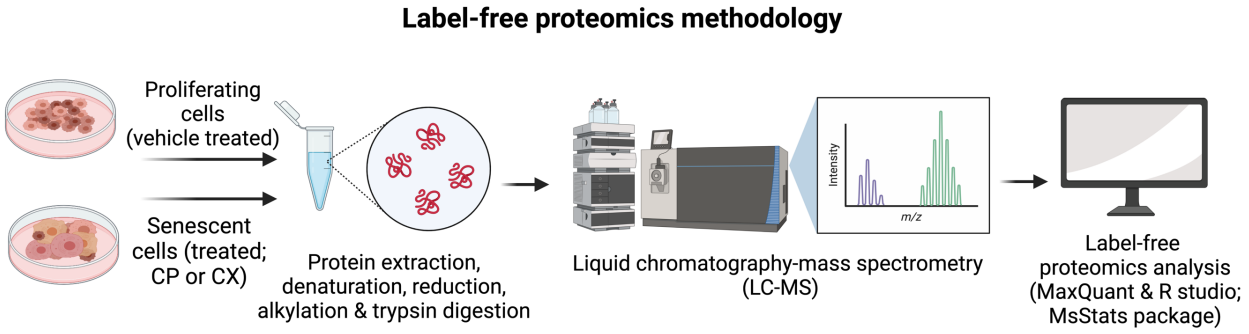

Figure 4. Label-free proteomics methodology.

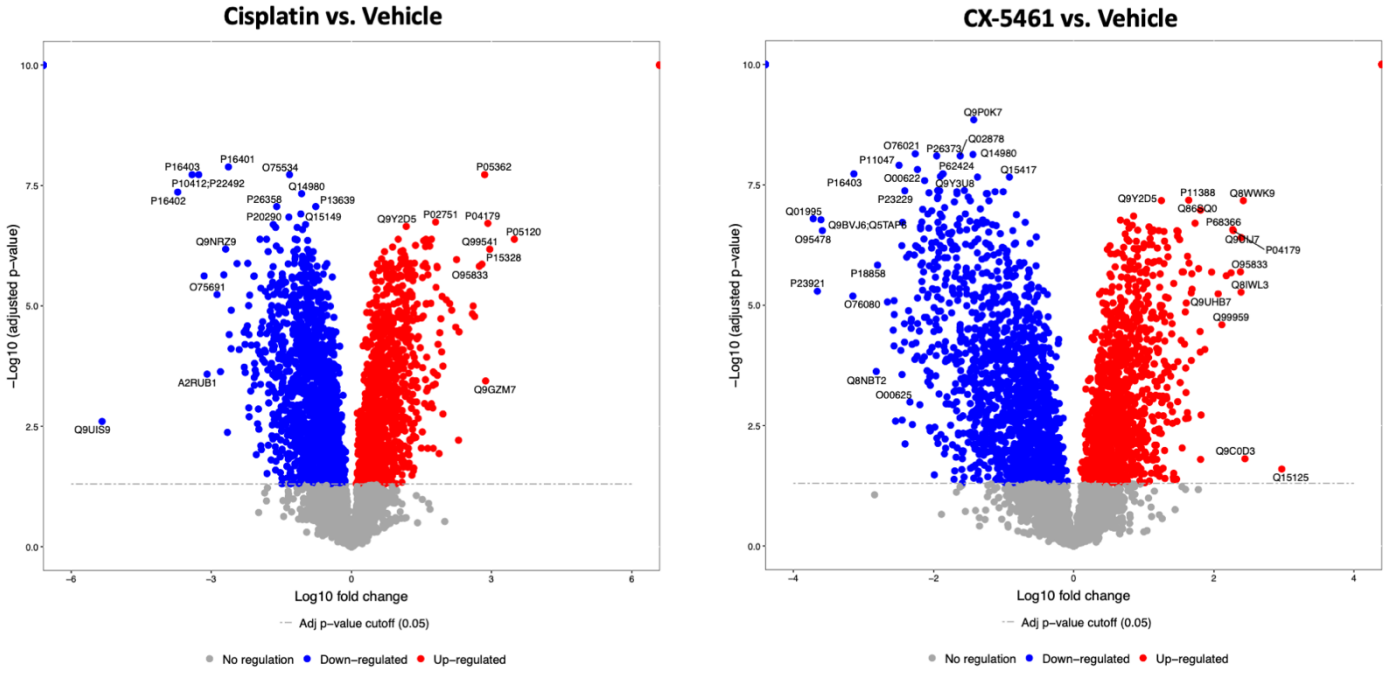

Figure 5. Volcano plots showing differentially upregulated (red) and downregulated (blue) proteins in OVCAR-8 cells.

Milestone #2: Identify cell surface proteins that can be potential targets of TIS for treating HGSC

Using data from generated from OVCAR-8 cells, we prioritized targets to test, based on differentially upregulated genes or proteins as determined by RNAseq and proteomics, to test in the boutique CRISPR/Cas9 knockout screen in Aim 2.

Specific Aim 2: Identify novel strategies to eradicate senescent HGSC cells (senolytics)

Major Task 1: Perform boutique CRISPR/Cas9 knockout screen using the TransEdit guide RNA library (3 vectors/gene), prioritised from analyses in Aim1, in OVCAR-8 cells. **We initially planned to use lentiviral guide RNAs, but we instead opted to use synthetic single guide RNAs to allow better for control of cell seeding number in a phenotypic screen. We have completed the screen in this format.**

Subtask 1: Optimize transduction in OVCAR-8 Cas9-expressing TIS cells and readouts (cell number, SA-β-gal). **COMPLETE**

Subtask 2: Transduce the library, collect cells and harvest genomic DNA at timepoints. **We revised our plan to instead perform a phenotypic screen.**

Subtask 3: Validate high-priority gene candidates in a secondary screen. **Commencing August 2023**
Subtask 4: Validate high-priority gene candidates in 2 immune-competent ID8 cell lines (*Trp53*$^{-/-}$/*Pten*$^{-/-}$, *Trp53*$^{-/-}$/*Nf1*$^{-/-}$). **Commencing October 2023**

Time course/workflow of screen

1. Seed cells for drug treatment
2. At 1 d post-seeding, treat with cisplatin or CX-5461
3. At 3 d post-seeding, perform drug washout and replace with fresh media
4. At 6 d post-seeding, replate cells into 384-well plate at 3500 cells/well and transfect with sgRNA.
5. At 14 d and 21 d post-replate/transfection, fix and stain cells.

Table 1. Gene targets tested in boutique CRISPR/Cas9 knockout screen.

ABCA4	ATF3	CCN3	CYC1	FAM213A	GPX1	IRF1	MMP1	ODR4	POU2F2	RP2	SLC25A3	TMEM158	VKORC1L1
ABCB4	ATP5J2	CCSMST1	CYLD	FAM43A	H2AFY	IRF7	MPST	OGDH	PPP1R15A	RPRD1B	SLC9A3R1	TMEM194A	VNN1
ABCC1	ATP5O	CD40	CYP20A1	FAM71F1	H2BC12	IRF9	MT1M	OLR1	PPP1R7	RRAGC	SLFN11	TMEM205	VNN2
ABHD12	ATP6V0A2	CD44	CYP4F3	FBLL1	H2BC8	IRGM	MTCH2	OPA1	PRAF2	RRAGD	SLFN13	TMEM52B	VPS11
ABHD2	ATP6V1E1	CD47	DAB2	FBXO32	H3C4	ISG15	MTOR	OPTN	PRCP	RRAS2	SNHG9	TMEM65	WDR26
ABRACL	ATPIF1	CDKN1A	DAGLB	FLOT2	HADHA	ITGB2	MTX2	PALLD	PRR11	RSAD2	SNORD13	TMEM70	WDR37
ACAA2	AVPI1	CHCHD4	DCK	FN1	HBEGF	JUN	MXRA8	PALM2	PRSS35	S100A8	SNRPG	TNFAIP3	WFDC21P
ACO2	B2M	CKAP2	DDAH1	FN3KRP	HELZ2	KIF11	MYDGF	PAM16	PSMB8	SAA1	SNRPGP15	TNFRSF9	WFDC3
ACOT1	BASP1	CKAP2L	DDI2	FOLR1	HERC6	KITLG	MYEF2	PAPSS2	PTGR1	SAA2	SOD2	TNFSF14	WNT10A
ACOT9	BCL2L13	CLCN7	DDX58	FOSB	HEXB	KLHL21	MYEOV	PARP12	PTK2B	SACM1L	SOST	TNIP3	XAF1
ACSF2	BCL6	CLGN	DDX60	FOXO1	HHEX	L2HGDH	MYH10	PARP9	PTPRR	SAMD9L	SP140	TNKS2-AS1	XDH
ADAMTS12	BET1	CLIC2	DHRS3	FTH1	HINT2	LACTB	MYL6B	PCDH1	PTRH2	SAMM50	SPATS2L	TOMM22	XPNPEP1
ADAMTS9	BIRC3	CLIC3	DHX58	G0S2	HK1	LACTB2	NAE1	PCDH19	PTX3	SAT1	SPECC1	TOMM40	YARS2
ADAR	BLOC1S3	CMAS	DIABLO	GADD45A	HMGCL	LAMB3	NAGK	PCDH7	PURA	SBSN	SPHK1	TOP2A	YBX2
ADO	BST2	CMC2	DLAT	GADD45B	HMMR	LAMC2	NAMPT	PCYOX1	PVRL3	SDC4	SPTBN1	TRAF1	YES1
ADPRHL2	C11orf54	CMPK2	DLST	GANAB	HNRNPUL1	LAMP2	NANS	PDHA1	QPRT	SDPR	SQSTM1	TRIM25	YPEL5
ADRB2	C14orf142	CNN1	DMBT1	GAR1	HRH1	LCMT1	NAPSA	PDHX	RAB11FIP5	SEC22B	SRGN	TRIM5	ZBP1
ADTRP	C15orf48	CNNM3	DNAJB6	GATA6-AS1	HRK	LCN2	NCCRP1	PDIA5	RAB13	SEC23B	STAT1	TRNL1	ZC3H12A
AFF4	C19orf47	CNNM4	DNAJC19	GBA	HRSP12	LCP1	NDUFAF2	PDPR	RAB29	SEC24A	STAT2	TSACC	ZC3H12C
AFG3L2	C19orf52	CNP	DTX3L	GBP4	HSCB	LETM2	NEAT1	PDZK1IP1	RAB3B	SEC31A	STK38	TSC22D1	ZCCHC6
AFP	C2CD2L	COG2	DUSP10	GBP5	HSD17B10	LGALS1	NEDD4L	PEA15	RAB5A	SEC63	STOML2	TSN	ZFAND2A
AIFM1	C2orf47	COL7A1	DUSP4	GBP6	HSD17B12	LIMCH1	NEK9	PGRMC1	RAB8B	SELENOF	STX12	TSPOAP1-A	ZNF107
AK1	C3	CP	DUSP5	GGCT	HTRA2	LIMS3	NEU1	PGRMC2	RAB9A	SERPINB1	STX3	TUBA4A	ZRANB2
AK3	C4A	CPA4	DUSP6	GGH	ICAM1	LIMS3L	NEURL3	PHB	RAD50	SERPINB2	STX7	TUBB2A	ATP1B3
AKAP2	C4B	CPEB4	EFNA1	GGPS1	IDH3A	LMCD1	NEXN	PHF11	RALA	SERPINB6	STXBP6	TUBB2B	ZNF436
AKAP8L	C4orf19	CRABP2	EIF2AK2	GJB3	IDH3B	LMO7	NFKB2	PHLDA1	RAP2B	SERPINB8	SUPV3L1	TUFM	MYD88
AKR1C1	C7orf61	CRACR2B	EMC3-AS1	GLCCI1	IFI35	LNPEP	NFKBIA	PHLDB2	RAP2C	SERPINB9	SWAP70	TYMSOS	RBM28
AKR1C3	CAMK2D	CSF1	EML4	GLIPR2	IFI44	LPAR1	NFKBIZ	PI3	RASAL2-AS1	SERPING1	TACC3	UBA3	GPR137C
ALDH7A1	CAMK2G	CSRP2	ENC1	GLRX	IFIH1	LRPPRC	NFXL1	PI4K2A	RASGEF1A	SERTAD4-AS	TANC2	UBE2L6	EYS
AMZ1	CANX	CTSE	ENO2	GMPR2	IFIT1B	LSR	NIT1	PITHD1	RBBP6	SFXN1	TCIM	UPP1	SERINC3
ANGPTL4	CASC8	CTSS	EPAS1	GNAI3	IFIT3	LYPLAL1	NPC1	PIWIL2	RBP7	SGK1	TCOF1	UQCC1	TRIM33
APOOL	CAV1	CUTA	EPB41L2	GNB4	IFRD1	MACF1	NPC2	PKP2	RDH11	SHOX2	TFAM	UQCC2	ATP1A3
AREG	CBX5	CXADR	EPB41L5	GNG12	IGF2BP2	MAP1LC3B	NRIP3	PLA2G4C	RDH14	SLC12A2	TGFA	UQCR10	PRR36
ARF6	CCDC51	CXCL1	EPS8	GNGT2	IGF2BP3	MATN2	NT5DC3	PLAT	RELB	SLC16A12	TGM2	UQCRC2	RUNX1-IT1
ARL8B	CCDC58	CXCL10	ERGIC1	GNS	IL1A	MCAM	NUDCD2	PLEKHF1	REPS1	SLC25A1	TIMM10	USO1	HAGHL
ARMCX3	CCDC90B	CXCL3	ETFA	GOT2	IL32	MCCC1	NUP210	PLEKHO2	RER1	SLC25A11	TIMM10B	USP18	ZNF568
ARMT1	CCK	CXCL5	ETFB	GPD2	IL4R	MDC1	OAS2	PLIN2	RHOC	SLC25A12	TIMM8A	VAMP7	FCER1G
ASAH1	CCL2	CXCL8	FAF2	GPNMB	IL7R	MDH2	OAS3	PML	RHOU	SLC25A13	TIMM9	VDAC2	HMX2
ATAD3A	CCL20	CXCR4	FAHD1	GPR87	IMPA1	MECP2	OASL	PNPLA6	RMND1	SLC25A19	TLR3	VGLL3	HYDIN
ATAD3B	CCL26	CYB5B	FAM136A	GPRC5A	INHBA	MGME1	OCC1	POLG2	RNF213	SLC25A20	TMCO1	VKORC1	RFESD

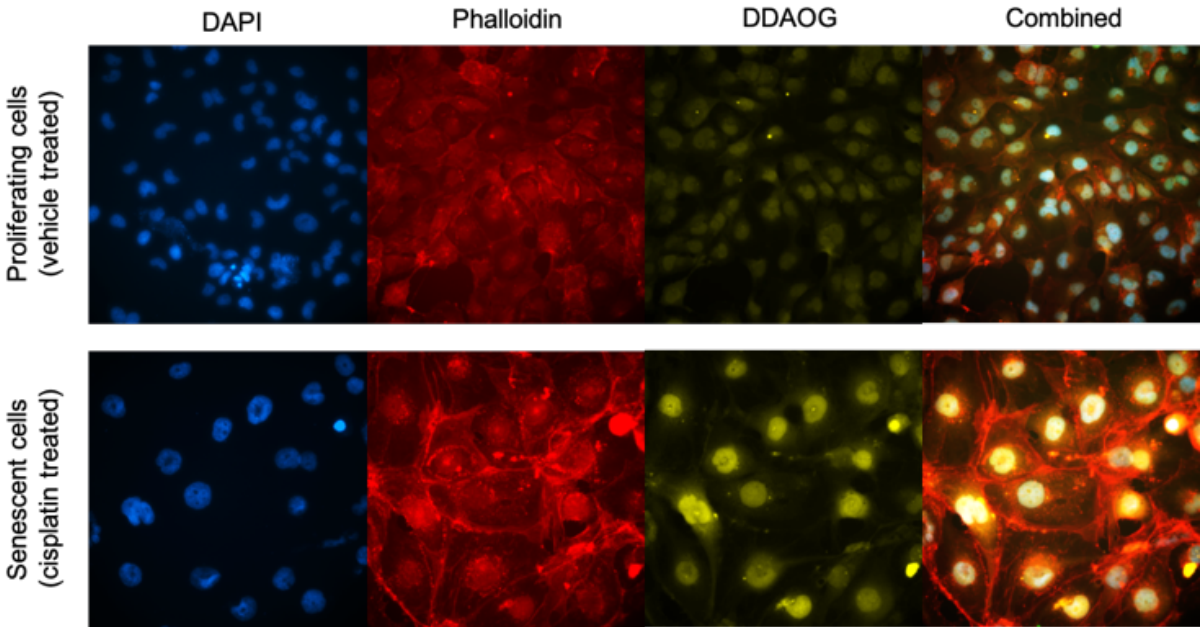

Figure 6. Example images for screen readouts of cell number (DAPI), size (phalloidin) and SA-beta-gal activity (DDAOG) in proliferating vs. senescent cells.

Milestone #3: Identify the high-priority gene targets for the identification of novel senolytic drugs

We have successfully completed a boutique CRISPR/Cas9 knockout screen in which we tested a total of 560 genes prioritized from differentially upregulated genes/proteins observed in the RNAseq and proteomics data. We identified a number of gene targets, which when knocked out, resulted in either increased cell number (escape from TIS) or decreased cell number (senolytic). We are validating targets on either end of this spectrum.

In particular, we have identified the gene target *FTH1* (ferritin heavy chain 1) as a high-priority gene target for follow-up as a senolytic strategy. FTH1 upregulation during TIS was confirmed by Western blotting in all cell lines described. FTH1 is a key enzyme regulator of iron homeostasis and its overexpression has been shown to inhibit ferroptosis. Ferroptosis is an iron-dependent and lipid peroxidation dependent mechanism of cell death, distinguishing it from apoptosis. The induction of ferroptosis could therefore be a potential strategy to eradicate TIS cancer cells.

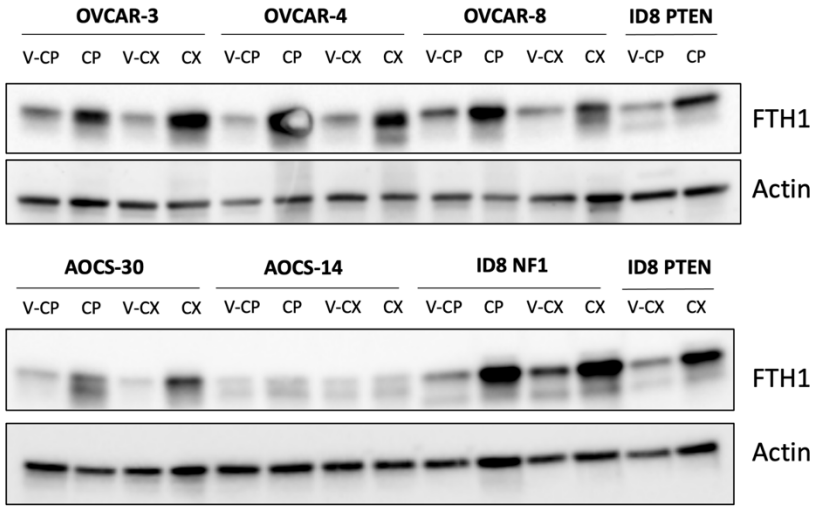

Figure 7. Western blots showing upregulation of FTH1 in TIS in established HGSC cell lines, patient-derived cell lines and murine ovarian cancer cell lines.

Major Task 2: Perform compound screens in OVCAR-8 cells to identify senolytic drugs. We have p

Subtask 1: Optimise high-content readouts for screen (cell number, SA-β-gal, cleaved Caspase 3/7, conditioned media for ELISA). **COMPLETE**

Subtask 2: Screen FDA-approved compound library

Subtask 3: Screen apoptosis library

Subtask 4: Screen epigenetic library

We have successfully performed drug screens of 2322 compounds from the Compounds Australia library. These compounds comprise FDA-approved, apoptosis, and epigenetic compounds.

OVCAR8-H2B-GFP senescence screen workflow using Cisplatin/CX-5461 as senescence inducers:

Day 0: Plate 3E^6 OV8-H2B-GFP cells into T175 flasks.

Day 1: Treat the OV8-H2B-GFP cells with senescence inducing drugs (cisplatin 10 uM or CX-5461 3 uM).

Day 3: 48h post drug treatment, lift cells with PBS + 10 mM EDTA, count and seed (3500 CX-5461 or 5000 Cisplatin) cells per well into 384 well platesin VCFG using a Biotek EL406 dispenser.

Day 9: (6 days post washout/Biotek cell seeding) replace media with fresh RPMI complete media and dispense control compounds and Incucyte red viability dye (1:8000) using the Tecan D300e Digital Dispenser. Next dispense the Compounds Australia Apoptosis library at 5 uM and 1 uM using the Janus Robot. The plates are then imaged using an Incucyte SX5 live cell imager every 4 h for 24 h.
The controls compounds used for this screen are as follows:
ABT-263 (50 uM, 20 uM, 10 uM, 5 uM, 3 uM, 1 uM, 0.1 uM)
ABT-737 (50 uM, 20 uM, 10 uM, 5 uM, 1 uM, 0.1 uM)
A-1153 (50 uM, 20 uM, 10 uM, 5 uM, 1 uM, 0.1 uM)
Mitomycin (100 uM, 10 uM, 1 uM, 0.1 uM)
Staurosporine (50 uM, 10 uM, 1 uM, 0.1 uM)

Day 10: 24h post drug treatment fix plates with 4% PFA and stain with DAPI 2 ug/mL and 1X Rhodamine-Phalloidin (PBS + 0.3% Triton X-100). Image plates on the Cellomics CX7 LZR platform at 10X magnification.

Readouts for analysis:
Incucyte: Greed and red cell object counts to observe viability (nuclear GFP) and cell death over the 24h.
Confocal images: using analysis pipeline looking where only DAPI nuclei with a minimum cell area (based on phalloidin staining) will be counted compared to cell death seen with senolytic ABT-263 at 5 uM and 1 uM.

Compounds Australia plate map template (compounds in purple only):

DESTINATION Layout - PMC161_2ptCP

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20	B21	B22	B23	B24
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
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I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I14	I15	I16	I17	I18	I19	I20	I21	I22	I23	I24
J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14	J15	J16	J17	J18	J19	J20	J21	J22	J23	J24
K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18	K19	K20	K21	K22	K23	K24
L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24
M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24
N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16	N17	N18	N19	N20	N21	N22	N23	N24
O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12	O13	O14	O15	O16	O17	O18	O19	O20	O21	O22	O23	O24
P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24

Sample Vol (nL)			200	40	200	40	200	40	200	40	200	40	200	40	200	40	200	40	200	40	200	40	0	0
BF Vol (nL)			0	160	0	160	0	160	0	160	0	160	0	160	0	160	0	160	0	160	0	160		
Total Vol (nL)	0	0	0	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	0	0

Controls dispensing plate map:

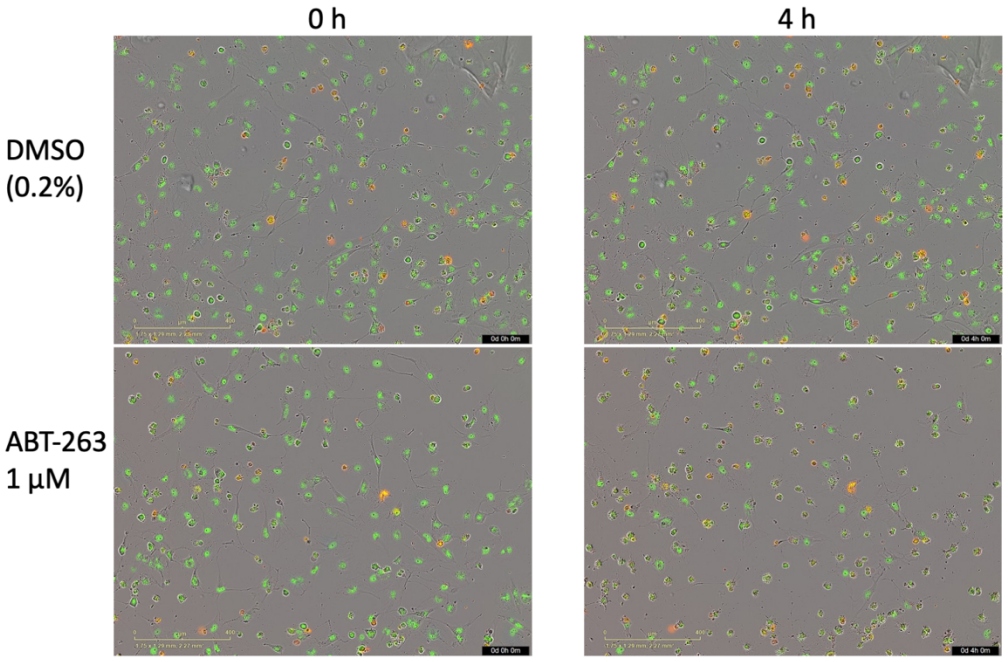

Figure 8. Incucyte images of OVCAR-8 cisplatin TIS cells marked with H2B-GFP (nuclear marker) and Cytotox Red (cell death dye).

Milestone #4: Discover novel senolytic drugs to eradicate TIS HGSC cells to be tested in vivo

We have analyzed the data from primary screen to identify novel senolytic drugs killing TIS HGSC cells. We have identified 52 compounds with a Z-score < -3, which show a marked fold change decrease in cell viability, indicating robustness of the screen design to be able to identify candidate senolytic targets. Many appear to be more potent than the known senolytic ABT-263/Navitoclax, which we included as a control in our screen design. We will perform a secondary screen using these drugs with a wider range of doses to demonstrate dose-dependent effects of these compounds.

Major Task 3: Validation of senolysis in patient-derived organoids (AOCS-20). **Commencing October 2023.**

Milestone #5: Establish novel senolytic drugs for treating HGSC. We are already validating a few candidate drugs from the primary screen and will validate highest priority drugs following the secondary screen in other cell lines to be updated in the next reporting period.

Specific Aim 3: Identify approaches to re-instate TIS in vivo

Major Task 1: In vivo CRISPR/Cas9 knockout screen in ID8 (*Trp53*$^{-/-}$/*Pten*$^{-/-}$, *Trp53*$^{-/-}$/*Nf1*$^{-/-}$) lines transplantable into C57Bl/6 mice. **We have revised our plan to instead perform a CRISPR/dCas9 activation screen. The rationale for this is that the overexpression of genes that result in enhanced tumour growth would be candidate genes causing TIS escape. Inhibitors against these would be potential strategies for re-instating TIS in vivo.**

Subtask 1: Optimize genome-wide library transduction in ID8 Cas9-expressing, senescence reporter TIS cells. **We ran into issues with the senescence reporter, but we have optimized conditions to obtain a complete population of TIS cells. We have successfully optimized lentiviral transduction of the genome-wide CRISPR activation sgRNA library (Caprano) in TIS cells.**

Subtask 2: Perform in vivo screen in C57Bl/6 mice, harvest tumors and perform next-generation sequencing on tumor cells (n=20 mice). **Commencing September 2023**

Subtask 3: Analyze enriched sgRNAs. **Commencing November 2023**

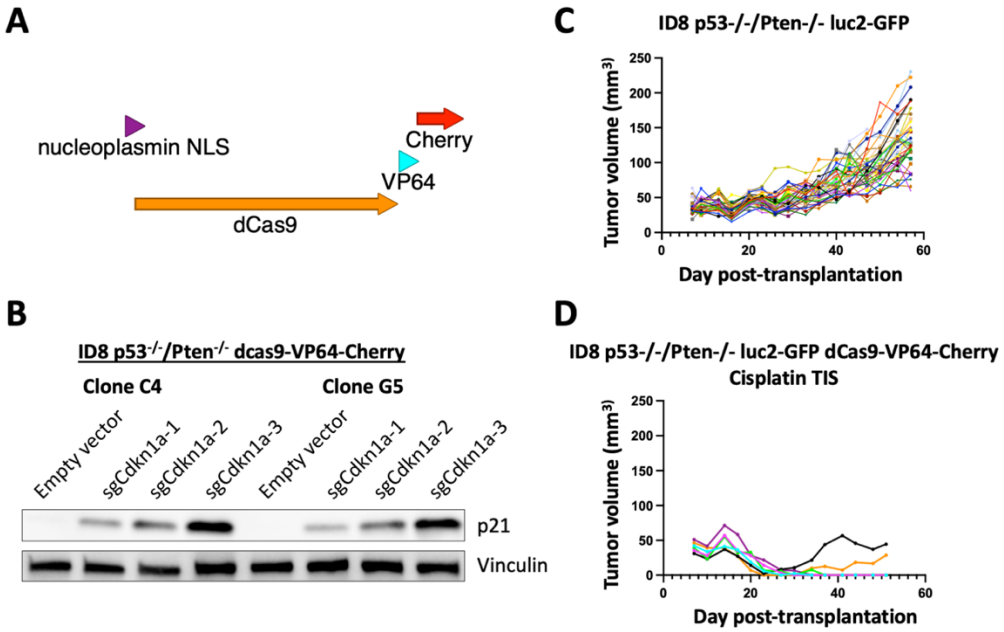

Figure 9. (A) Schematic of lentiviral dCas9-VP64-Cherry construct used to transduce ID8 p53-/-/Pten-/- cells. (B) cells expressing sgRNAs against p21 demonstrating successful p21 induction. (C) Tumour growth

from ID8 p53-/-/Pten-/- luciferase-GFP cells following subcutaneous implantation into n=40 mice. This experiment was from a non-DOD funded project but is used to demonstrate feasibility and kinetics of the revised mode of tumour cell implantation. (D) Tumour growth from cisplatin TIS ID8 p53-/-/Pten-/- luciferase-GFP cells expressing dCas9-VP64-Cherry following implantation into n=6 mice, demonstrating lack of growth compared to proliferating tumour cells in (C).

The experiments from Specific Aim 3 Major Task 2 and onward will be the major focus of the next reporting period.

Opportunities for training and professional development

Nothing to report

Dissemination of Results

Madeleine Tancock, my PhD student, presented her work related to Aim 1 of this proposal in the form of a poster at the International Cell Senescence Association Meeting held in Groningen, Netherlands (2022).

Title: Harnessing therapy-induced senescence to treat high-grade serous ovarian cancer

Authors: Madeleine Tancock, Keefe Chan, Jian Kang, Anna Trigos, Lachlan Cain, Richard Pearson

Abstract

DNA-damaging chemotherapeutic agents can prematurely induce the cellular senescence state in cancer cells, termed therapy-induced senescence (TIS). TIS in cancer cells has demonstrated reversibility and could contribute to disease relapse. High-grade serous ovarian cancer (HGSOC), has incredibly poor survival rates, characterised by high disease relapse and limited treatment effectiveness and options. Efforts to understand and develop better treatments for this disease are drastically sought. We examined the role of the TIS response via two genotoxic agents, cisplatin and a novel ribosome-directed drug CX-5461, in multiple HGSOC cell lines. We assessed the gene expression changes via bulk RNA-sequencing and examined the secretome, via antibody arrays, of these cells to better understand the impact of the TIS phenotype in HGSOC cells. Our results demonstrated that HGSOC cells that undergo TIS engage in an inflammatory stress-response phenotype. Targeting this inflammatory stress-response may provide an alternative therapeutic strategy to improve outcomes in this disease.

Goals of the next reporting period

We have made significant progress in the first reporting period with respect to the SOW. We are focusing our investigation on the key hypothesis that ferroptosis inhibition is a feature maintaining the survival of TIS HGSC cells, which can be exploited for therapy. The next phase of the project will predominantly involve performing experiments to validate this hypothesis in vitro and in vivo.

We have exciting data from the genetic screens and compound screens that have identified both senolytic and senescence reinstatement targets. Our goal is to validate these targets in vivo using the ID8 subcutaneous model.

Changes/Problems

Changes in approach and reasons for change

There have been no changes in the key approaches of the project. We have revised specific experiments due to technical challenges but we believe they have been beneficial and will facilitate progression of the project.

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

Nothing to Report

Significant changes in use or care of vertebrate animals.

We have put in an amendment to our previous animal protocol E706 to refine our model. Our increasing experience with the intraperitoneal injection model has informed us that this model enables us to reliably profile immune cells within the ascites fluid. However, it has been challenging to examine the behaviour of the tumour cells, as tumour burden and ascites development do not always correlate, causing mouse to mouse variability. This has prompted us to forego the use of the intraperitoneal injection model and instead use subcutaneous injection of tumour cells. **We believe this method of refinement to our protocol will help to ameliorate the variability by allowing us to more reliably examine senescence within a localised tumour and decrease the number of interventions to the mice.**

Significant changes in use of biohazards and/or select agents

Nothing to Report

Participants & Other Collaborating Organizations

What individuals have worked on the project?

In addition to the PI on the grant, the following people have worked on the project:
Carmelo Cerra – 60% Research Assistant listed as personnel on this project
Madeleine Tancock – PhD Student

Translational Research Centre (TRC) – To enhance the feasibility of completing the in vivo experiments detailed in Aim 3, we have enlisted the help of the TRC within our research division, which assists researchers in performing animal experiments according to their approved animals ethics protocols. Some salary has been transferred to the Research Assistants included on our animal protocol to perform implantations of tumor cells, treat and monitor mice.

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

Co-Investigator Pearson has retired from his position as Associate Director of Laboratory Research but retains an Honorary Group Leader Role at the Peter MacCallum Cancer Centre.

What other organizations were involved as partners?

Nothing to Report