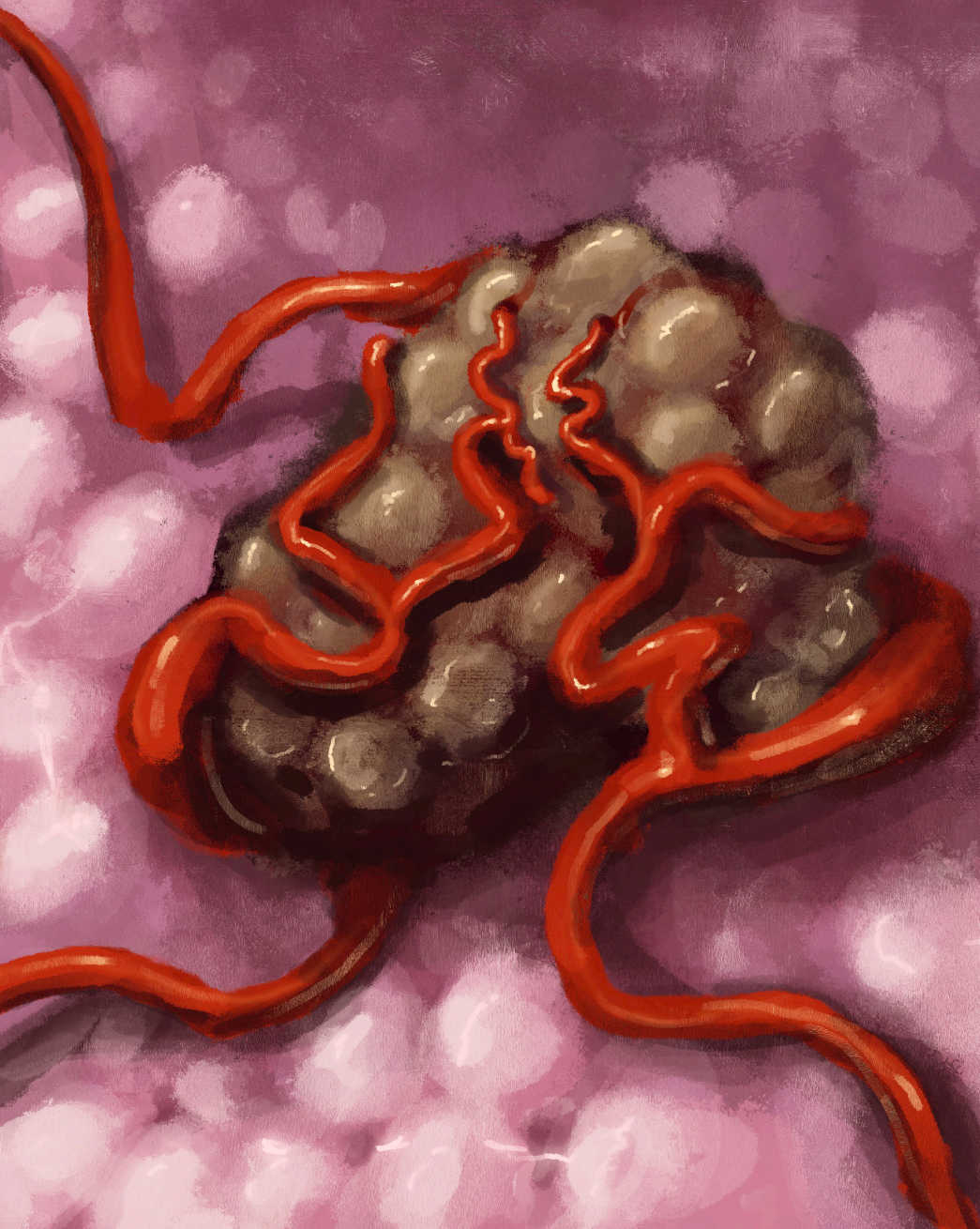


VERTICES

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Impact of Aged Tumor
Microenvironment and
Chemosensitivity on
Sub-types of Ovarian
Cancer

Identity, Resilience and
their Psychological
Impact Among Older
Adults with Cancer

Investigating a link
between Glyphosate and
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Beyond Existing
Analysis: The Rigorous
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The Influence of Cancer
Associated Fibroblasts
on Glioblastoma Tumor
Migration

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Letter from the Editor

Dear Reader,

We are delighted to welcome you to the second issue of our third volume, where we present five original research articles that have undergone rigorous peer review by our wonderful academic review team. This publication is similar in style and form to our previous issues, containing masterfully-crafted visuals and short article synopses to help readers from all backgrounds access these works.

This issue's focus is on cancer biology, with four out of our five articles presenting new research in this field. From basic-science studies on the role of the tumor microenvironment in cancer progression, to clinically-focused experiments aiming to test new chemotherapeutics, the data shown in these articles are a great way to gain some insight into ongoing questions in cancer biology. Additionally, we present a fascinating article that evaluates the classic debate over the extent to which humans have free-will from a neuroscience perspective. Between these five articles, there is a mix of experimental approaches, arguments, and perspectives that should satisfy the diverse interests of our readers.

As always, this issue is a product of the diligent effort of our peer review team, our artistic team, our collaborators at Georgetown University, and the gracious faculty, post-doctoral scholars, and graduate students who helped peer review these articles. Our team found these articles insightful and thought-provoking, and we hope you will too! Please enjoy the publication.

Sincerely,

A handwritten signature in black ink, appearing to read "Kaeden Hill". The script is fluid and cursive, with the first name being more prominent.

Kaeden Hill, Co-Editor in Chief

A handwritten signature in black ink, appearing to read "Sasha Bacot". The script is fluid and cursive, with the first name being more prominent.

Sasha Bacot, Co-Editor in Chief

Impact of Aged Tumor Microenvironment and Chemosensitivity on Sub-types of Ovarian Cancer

Lila Taylor, Dr. Zhiqing Huang



Article Synopsis

This study investigates how aging effects the growth of ovarian cancer, as well as sensitivity of different ovarian cancers to various chemotherapies. By growing and aging non-cancerous cells to mimic the environment ovarian cancer would see inside the human body, we were able to find that in the lab, ovarian cancer cells grew worse in the “older” condition compared to the “young” environment. Additionally, we identified a chemotherapy called ibrutinib that effectively targets slower growing ovarian cancer cell lines.



Graphic by AJ Kochuba

Impact of Aged Tumor Microenvironment and Chemosensitivity on Sub-types of Ovarian Cancer

Lila Taylor, Dr. Zhiqing Huang

<https://doi.org/10.55894/dv3.21>

ABSTRACT

Ovarian cancer (OC) is the most lethal female reproductive cancer—with a 5-year survival rate of 50.9%—and is diagnosed on average in patients in their 60s. Often OC patients enter an initial remission following chemotherapeutic or surgical interventions; however, ~75% of patients experience chemotherapy-resistant OC recurrence. This study aims to investigate the impact of aging on the tumor microenvironment (TME) and chemosensitivity of various subtypes of ovarian cancer (OC) to therapies targeting slower proliferating OC cells, which often lie dormant in patients and lead to disease recurrence. We have found slower proliferating OC cells exhibited a statistically significant higher sensitivity to the chemotherapy treatment ibrutinib compared to faster-proliferating cells in high-grade serous ovarian carcinomas (HGSOCs) in vitro. Cisplatin-resistant HGSOC cell lines displayed increased chemosensitivity to ibrutinib compared with the cisplatin-sensitive HGSOC cell lines. In clear cell ovarian carcinomas (CCOCs), we did not see dose-dependent chemosensitivity to duvelisib or ibrutinib, but there was an increased sensitivity when compared to the fast-proliferating HGSOC control. CCOC cell lines JHOC5 and TOV21-G showed increased responses to higher doses of UCN-01, but OVISe, the slowest proliferating CCOC did not. When looking at OC cell proliferation in conditioned medium from aged versus young WI-38 fibroblasts, OC cells were less proliferative in aged WI-38 medium when compared with the OC cells in young WI-38 medium in both HEYA8 and CAOV2 cell lines. Furthermore, we found that older WI-38 fibroblast coculture increased chemosensitivity for M41 HGSOC cells treated with duvelisib but not carboplatin. Future directions will be focused on the mechanisms of chemosensitivity in varying tumor microenvironments.

INTRODUCTION

Aging is a significant risk factor for many adult cancers, including ovarian cancer (OC). OC is the deadliest female reproductive system cancer, with a median diagnosis age of 63 years.¹ The 5-year survival rate for OC is 50.9%, according to the Surveillance, Epidemiology, and End Results (SEER) Data Set.² Most patients diagnosed with OC will go into initial remission following surgical debulking and adjuvant chemotherapy.³ However, approximately 75% of patients will experience a recurrence of OC, often characterized by cancer cells that have become resistant to initial chemotherapies.⁴ Chemotherapy resistance presents a significant issue for patients facing recurrent OC.

As the most common subtype of epithelial ovarian carcinoma, high-grade serous ovarian carcinoma (HGSOC) accounts for 70% of epithelial ovarian cancers and 90% of all ovarian cancers.⁵ HGSOC is the most lethal sub-type of ovarian cancer according to the data from the SEER data set in which patients were followed four years or longer after cancer detection and treatment. HGSOCs are generally composed of solid masses of cells and are characterized by high-grade serous epithelial cell atypia and TP53 mutations.⁶ It has been proposed that high-grade serous tumors originate from the epithelium of the fallopian tube.⁷ There are currently five principal histotypes of ovarian carcinoma: clear cell ovarian carcinoma (CCOC), HGSOC, low-grade

serous ovarian carcinoma (LGSOC), mucinous ovarian carcinoma (MOC), and endometrioid ovarian carcinoma (EOVC).⁸

HGSOCs tend to occur in older, usually menopausal women.⁹ About 80% of HGSOC cases are diagnosed at advanced stages, such as stage III and stage IV, when the tumors are aggressively growing and invading/migrating to the other organs in the peritoneal cavity or even the distant organs.¹⁰ At these stages, the tumors are most spread out, and patients no longer have the option of effective surgical treatment. There are a variety of contributors to the high mortality rate of HGSOC: the inability to diagnose HGSOC early on due to vague symptoms, the lack of efficient screening tests, and the high frequencies of tumor recurrence.^{6,11}

Tumors are comprised of cancer cells that carry the abnormal genetic and epigenetic information that contribute to cancer development and progression. Surrounding the tumor is the tumor stromal microenvironment, which is essential in supporting and regulating tumor growth and progression.¹² Over the last two decades, the focus of cancer research shifted from solely focusing on cancer cells to looking at the tissue more systematically and investigating the complex interactions between tumor cells and their surroundings—the tumor microenvironment (TME). The TME is comprised of both cellular and non-cellular components. Cellular components of the TME are tumor-associated fibroblasts (CAFs), endothelial cells, adipocytes, and immune cells, such as natural killer cells (NK), tumor-associated macrophages (TAM), tumor-associated neutrophils (TAN), myeloid-derived suppressor cells (MDSC), and adaptive immune cells like T cells and B cells.¹³ The non-cellular TME consists of proteins, glycoproteins, and proteoglycans, which make the extracellular matrix (ECM).¹⁴

While many studies have focused on the immune cells in TME, the importance and regulation of non-immune stromal components of TME have caught researchers' attention. The non-immune tissues are composed of stromal cells, such as CAFs, tumor endothelial cells (TEC), adipocytes, and the ECM.

Among non-immune stromal cells, CAFs are

among the most abundant and significant in TME tissues.¹⁵ Compared to regular fibroblasts, CAFs are more heterogeneous and produce more collagen, ECM proteins, and pro-tumor factors. Aided by CAFs' production of enzymes, crosslinking leads to various interactions between ECM components and tumor stroma stiffness, making conditions more favorable for invasion via malignant cells during ECM remodeling. This tumor stroma stiffness can cause blood vessel collapse at the affected site and consequent hypoxia, thus impairing drug delivery and inducing the proliferation of cancer cells. Additionally, CAFs release growth factors and cytokines that can promote tumorigenesis.^{16,17}

WI-38 fibroblast cells have been selected and used to model an aged TME. WI-38 cells are a human diploid lung fibroblast cell line with a finite lifetime that can be aged via passage in cell culture.¹⁸ Senescence of cells that rely on proliferation to thrive, such as stem cells or immune cells, are limited in their capacity to repair in aging tissues; senescence in WI-38 cells has become associated with reduced DNA methylation leading to differential gene expression.¹⁸ By culturing OC with WI-38 cells, or in WI-38 medium, we can model an aged TME to examine how age affects cell proliferation and chemosensitivity *in vitro*.

Disease recurrence is often observed in patients with platinum-resistant OC, due to low response rate¹⁹ and the ability for platinum-resistant, slow-growing OC cells to lie dormant for years following initial platinum-based chemotherapy treatment, such as cisplatin or carboplatin. Thus, it is crucial to identify potential drug candidates that could increase the efficacy of anti-OC treatments, targeting fast and slow-growing cells, as well as platinum-sensitive and resistant cells within a patient. Analysis of the NCI-60 dataset²⁰ revealed that chemotherapies duvelisib, ibrutinib, and UCN-01 were more efficient at targeting slow-proliferating cell lines, while CRB was more apt at targeting fast-proliferating cell lines.^{21,22} Drugs more effective on slow-proliferating OC could be used to target dormant cells, which are potentially the source of cancer recurrence.

In this study, we have tested the chemotherapeutic drugs within aged TME using the

WI-38 model. We examined the sensitivity of various sub-types of ovarian cancer to chemotherapies duvelisib, ibrutinib, and UCN-01. HGSOCS, ovarian cystadenocarcinoma, serous ovarian cancer with undetermined grade, and CCOCs were all studied in monoculture. Ibrutinib is an oral Bruton's Tyrosine Kinase (BTK) inhibitor, which in recent years has been used to treat hematological malignancies.²³ There are currently few published studies regarding the efficacy of ibrutinib in OC, as either a monotherapy or a polytherapy and chemosensitizer. However, recent clinical developments have revealed that ibrutinib may have efficacy in LGSOC.²⁴ UCN-01, a potent protein kinase inhibitor, shows selectivity for protein kinase C (PKC), which is a key component in the proliferation of OC via a signaling cascade involved in tumorigenesis.²⁵ Duvelisib is an oral phosphoinositide-3-kinase (PI3K) delta/gamma inhibitor, which currently is approved as a monotherapy for chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL).²⁶ AKT and mTOR, serine/threonine kinases that impact cancer cell cycling, metabolism, survival and growth,²⁷ work in tandem to form the PI3K/AKT/mTOR pathway, which is a frequently modified signaling pathway in OC.¹⁹ Carboplatin (CRB) is a second-generation platinum chemotherapy drug used as a high-dose treatment for aggressive OC but eventually produces drug resistance during treatment.²⁸

The test aims of this study were to examine the chemosensitivity of various HGSOCS and CCOCs to duvelisib, ibrutinib, and UCN-01. We then tested HGSOC cell proliferation in a comparison between the aged versus young TME, modeled by WI-38 medium. An initial study has been performed to evaluate the chemosensitivity of HGSOC in the aged and young WI-38 model TMEs to duvelisib, UCN-01, and CRB. Progesterone is a steroid hormone synthesized by the ovaries, corpus luteum, and placenta (J. D. Graham & C. L. Clarke, 1997) and plays a central role in the regulation of biological processes throughout uterine tissues: the two major layers of the uterus, the endometrium and the myometrium. The myometrium is the smooth muscle component of the uterus that protects the

developing fetus throughout gestation and generates contractile forces at the time of labor to deliver the fetus (Carsten, 1968). The endometrium forms the inner lining of the uterus, composed of the outer epithelium and inner stromal compartment. The epithelium thickens in anticipation of pregnancy and is the site of implantation if pregnancy is established. In the absence of pregnancy, menstruation occurs, resulting in the shedding of the endometrial lining (Critchley et al., 2020). If pregnancy is established, progesterone signaling triggers the differentiation of the stromal compartment into decidual cells (a process known as decidualization) that support embryo growth and maintain early pregnancy (Ng et al., 2020). Progesterone signaling is central to the two major layers of the uterus, the endometrium and the myometrium, regulating a host of biological processes including menstruation, ovulation, embryo implantation, uterine growth, and labor (J. D. Graham & C. L. Clarke, 1997).

MATERIALS AND METHODS

HGSOC & CCOC Culture, Seeding, and Chemotherapy Treatment

The OC cell lines were cultured in RPMI medium with 10% fetal bovine serum (FBS) and penicillin-streptomycin (PS) at 37°C, 5% CO₂. OC cell lines tested are listed in Table 1. Cell lines were seeded at 6000 cells per well into a 96-well plate for a 24-hour 37°C culture period. Cell lines were then treated with variable and increasing concentrations of ibrutinib, duvelisib, and UCN01, diluted with RPMI, and cultured for 72 hours at 37°C before 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay.

WI-38 Senescence

WI-38 fibroblast cell line was obtained from Duke Cell Culture Facility with initial population doubling times (PDTs) of 29 and an initial passage of p19. The WI-38 cells were cultured with Minimum Essential Medium Eagle (MEME) with 10% FBS/PS at 37°C, 5% CO₂, and passed when they reached 100% confluence. WI-38 cells were cultured over three months to obtain a

population-doubling level characteristic of an aged cell line. PDT was calculated using the formula $PDT = PDT_0 + 3.322(\log C_f - \log C_i)$.²⁹ In this formula, PDT_0 = initial population doubling level, C_f = final cell yield at the end of the growth period, and C_i = initial cell number seeded into the vessel.

M41/WI-38 Co-culture with Chemotherapy Treatment

M41 was selected for culture as it was derived from a patient with HGSOc with a slow proliferating feature with a PDT of 40.47 hours (Table 1). M41 was cultured with RPMI medium with 10% FBS/PS and seeded at 10,000 cells per well onto 12-transwell plates bottom chamber and incubated for 24 hours at 37°C, 5% CO₂. Aged (p29 and PDT 36) and young WI-38 (p19 and PDT 29) cells were seeded onto transwell plate upper chambers, allowing nutrient exchange between fibroblasts and OC lines, mimicking the TME in human patients. The co-cultured cells were incubated at 37°C, 5% CO₂ for 72 hours. After 72 hours of co-culture incubation, M41 cells in both young and old WI-38 conditions were treated with high and low doses of chemotherapies: duvelisib, CRB, and UCN-01. Chemotherapy-treated plates were incubated for 72 hours at 37°C, 5% CO₂. The upper chambers containing WI-38 fibroblasts were removed, and M41 cells in the bottom chamber were trypsinized and re-seeded into 96-well plates to use the plate reader for MTS assay, with six wells per treatment and control conditions. Relative cell viability was measured with MTS assay kit according to the protocol from the manufacturer (Promega).

HEYA8 & CAVOV2: WI-38 Co-culture

In Trial 1, WI-38 cell lines with PDT 36 and PDT 46 were used for young and old models respectively. 2.6×10^5 WI-38 cells were plated into 10cm diameter culture Petri-dishes and cultured until 70% confluence in MEME 10% FBS/PS at 37°C, 5% CO₂. The medium from WI-38 cell culture was collected. HEYA8 and CAOV2 cells were each seeded at 2000 cells/well into 96 well plates and cultured in both old and young WI-38 medium. The old and young WI-38 medium in the 96 well plates was replaced with fresh WI-38 medium and cultured for an additional

48 hours. After a total 96-hour culture period, cell viability was assayed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) kit according to the protocol from the manufacturer (EMD Millipore Corporation).

In Trials 2 and 3, WI-38 cell lines with PDT 34 (36 in Trial 3) and 55 were used for young and old models respectively. 6.25×10^5 WI-38 cells were plated into 10cm diameter culture dishes and cultured until 70% confluence in MEME 10% FBS/PS at 37°C, 5% CO₂. HEYA8 at 2000 cells/well and CAOV2 cells at 4000 cells/well were each seeded into 96 well plates and cultured in either old or young WI-38 medium. After the 48-hour incubation, the old and young media in the 96-well plates were replaced by the fresh WI-38 medium. After a total 96-hour culture period, cell viability was assayed using the MTT kit.

Cell Viability Test

The MTS or MTT assays were used to measure relative cell number for proliferation studies. MTT solution was added to each tested well on the 96 well plate and incubated at 4 hours at 37°C, 5% CO₂. Isopropanol was added to each well per the instruction manual on the MTT kit (Colorimetric [MTT] Kit for Cell Survival and Proliferation, EMD Millipore Corporation; 2015). For Trial 3 of the HEYA8/CAOV2 Aged vs. Young WI-38 co-culture experimentation, the plate was allowed to mix gently for an additional 20-minute period to better dissolve precipitate on the bottom of the wells. Absorbance was recorded at 570 nm using FLUOstar Omega Microplate Reader. For the MTS assay, CellTiter 96® Aqueous One Solution Reagent was added to each well³⁰, and an MTS assay was performed after a 1 hour 37°C incubation to measure relative cell number. Absorbance was recorded at 450nm using FLUOstar Omega Microplate Reader.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (v.9, GraphPad Software, Inc., La Jolla, CA, USA) or Microsoft Excel statistical programs. Statistical comparisons were made using a paired t-test for paired data or a two-way ANOVA testing with multiple comparisons, such as chemotherapy and

conditioned culture. A significance level of 0.05 was considered statistically significant.

RESULTS

Ibrutinib, Duvelisib, and UCN-01
 Chemosensitivity in OC Subtypes

Table 1: OC Cell Lines Used in The Study And Their Population Doubling Time (PDT)

Cell Line	Histology	PDT (hrs)
SK-OV-3	NS	19.49
HEY	HGSOC	20.53
JHOC5	CCOC	30 ³¹
TOV21-G	CCOC	30.62
OVCA432	HGSOC	38.52
M41	HGSOC	40.47
TYKNU	HGSOC	45
M41-cisR	HGSOC	45.41
OVISE	CCOC	48 ³¹
PEO4	HGSOC	52.34
OVCAR3	HGSOC	53.77
TYKNU-cisR	HGSOC	—

Listed above are all OCs used in the following experimentation: High-Grade Serous Ovarian Carcinomas (HGSOC), Clear Cell Ovarian Carcinoma (CCOC), and non-serous (NC).

We predicted that the selected drugs (UCN-01, duvelisib, and ibrutinib) would more effectively target the slower growing OC cell lines (ie. cell lines with larger PDTs). Additionally, we predicted that an aged TME would cause increased OC proliferation, due to the incidence of cancer progression and development in older populations. The cell lines used in this study together with their population doubling time (PDT) are listed in Table 1. In initial experimentation, we compared chemosensitivity to ibrutinib with 8 OC cell lines. M41 (PDT = 40.47) and M41-cisR (PDT = 45.41) show consistent results with increased ibrutinib sensitivity in cisplatin-resistant cell lines (Figure 1A). Treatment with duvelisib showed a decreased HGSOC cell viability and survival with a higher drug concentration, with the exceptions of TYKNU-cisR and HEY, which showed weaker response or even no response to duvelisib (Figure

1B). CCOC TOV21-G additionally did not show a strong response when treated with duvelisib. M41 showed a stronger response to duvelisib than M41-cisR (Figure 1B).

With ibrutinib, there was a statistically significant trend ($r = 0.82$, $p = 0.0128$) of slower proliferating cells exhibiting higher sensitivity to chemotherapy treatment than more rapidly proliferating cells (Figure 2A). Unlike ibrutinib, with duvelisib, there is a weak correlation ($r = 0.16$, $p = 0.6777$) of slower proliferating cells exhibiting higher sensitivity to chemotherapy treatment than fast proliferating cells (Figure 2B).

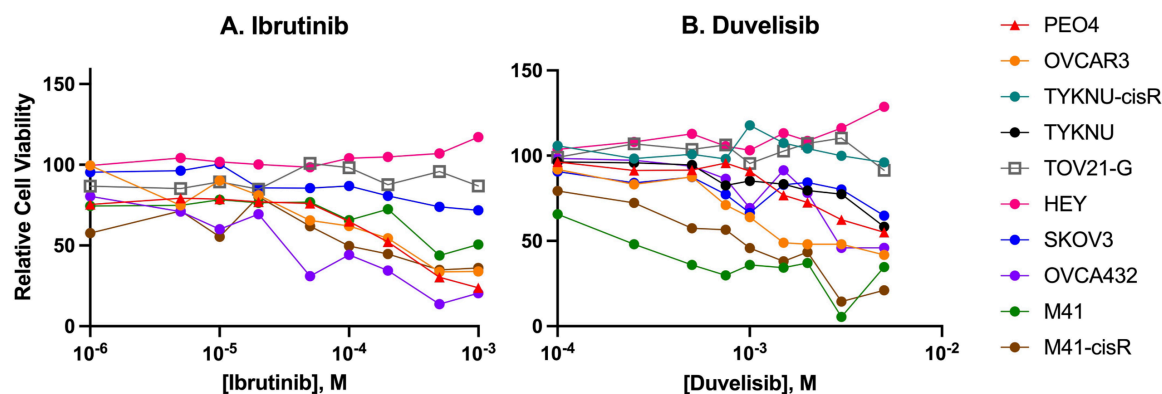


Figure 1. Chemosensitivity of OC cell lines to Ibrutinib and Duvelisib. Cell lines were plated into 96-well plates at 6000 cells per well and treated with drug for 72 hours. Readout performed with Cell Titer Glow MTS Assay. **A.** Slower proliferating cell lines are more sensitive to ibrutinib. CCOC (TOV21-G) and SOC (SKOV3) tested with ibrutinib showed weaker responses when compared to most HGSOC lines, excluding fast proliferating HGSOC HEY. M41-cisR showed a stronger response to ibrutinib than M41. **B.** No correlation between OC cell line sensitivity to duvelisib and PDT. Treatment with duvelisib showed increased HGSOC sensitivity at higher drug concentrations, with the exceptions of TYKNU-cisR and HEY, which showed weaker responses to duvelisib. CCOC (TOV21-G) did not show a strong response when treated with duvelisib. M41 showed a stronger response to duvelisib than M41-cisR.

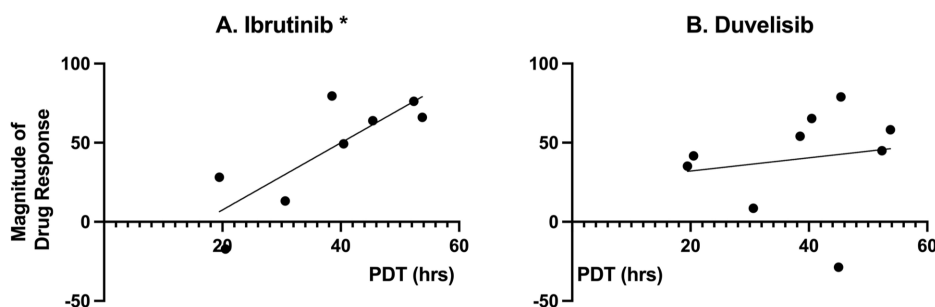


Figure 2. Positive correlation between the magnitude of relative drug responses and population doubling time (PDT) in Duvelisib and Ibrutinib conditions. Magnitude of relative drug response was defined as the greatest sensitivity from experiments in Figure 1A-B, with standard PDTs from Table 1, and each data point representing a different cell line for which we have chemosensitivity data and PDT data. **A.** With ibrutinib, slower proliferating cells exhibit a statistically significant ($p = 0.0128$) higher sensitivity to chemotherapy treatment than fast proliferating cells ($r = 0.82$). **B.** With duvelisib, slower proliferating cells exhibit higher sensitivity to chemotherapy; however, the correlation ($r = 0.16$) of slower proliferating cells exhibiting higher sensitivity to chemotherapy treatment than fast

An initial test was performed to test the chemosensitivity to ibrutinib (A), duvelisib (B), and UCN-01 (C) with 3 CCOC (JHOC5, OVICE, and TOV21G). We included the HGSOC cell line, HEYA8, as a comparison control. With the drug dose range for ibrutinib and duvelisib, we did not see dose-dependent effects, with prominent cell survival even when treated with high drug concentrations (Figure 3A-B). However, we have found the CCOC cell lines

were more sensitive to ibrutinib and resistant to duvelisib compared with HGSOC HEYA8 cells (Figure 3A-B). The mixed feature was seen with UCN-01 treatment, in which the CCOC JHOC5 and TOV21-G show increased responses to UCN-01 compared to HEYA8 (Figure 3C). More tests of CCOC lines may be needed to confirm the sensitivity to duvelisib and ibrutinib compared to HGSOC using the appropriate drug doses.

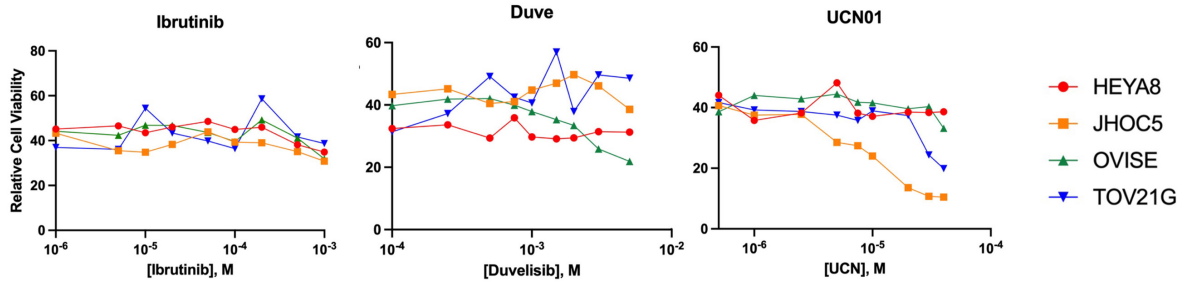


Figure 3. No response seen in CCOC when treated with Duvelisib and Ibrutinib compared to HGSOc. A. CCOCs (JHOC5, OVI5E, TOV21G) did not show a greater drug response to ibrutinib doses ranging from 10^{-6} M to 10^{-3} M when compared to insensitive HGSOc control HEYA8. **B.** Additionally, CCOCs did not show a greater drug response to duvelisib doses ranging from 10^{-4} M to 5×10^{-3} M. **C.** However, CCOC cell lines JHOC5 and TOV21-G show increased responses to higher doses ($\geq 10^{-5}$ M) of UCN-01 when compared to HGSOc HEYA8.

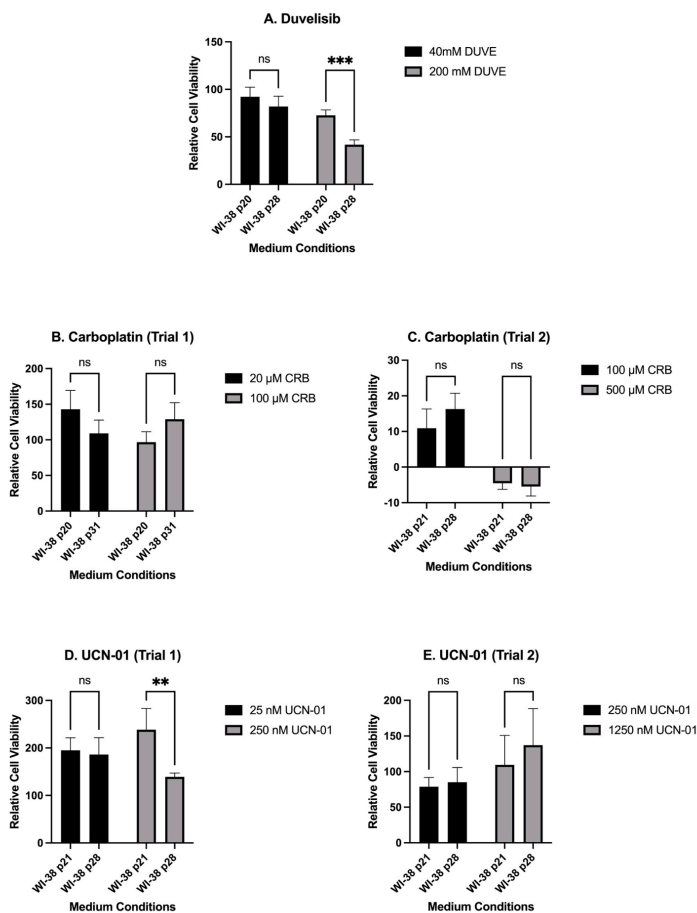


Figure 4. Older WI-38 fibroblast coculture increases chemosensitivity for M41 OC cells treated with high dose Duvelisib but not Carboplatin; results inconclusive for UCN-01 chemotherapy. In Trial 1, CRB low-dose and high-dose conditions were 20 μ M and 100 μ M, respectively. In Trial 2, CRB low dose and high dose conditions were 100 μ M and 500 μ M, respectively. For Trial 1, UCN-01 low dose and high dose conditions were 25nM and 250 nM, respectively. UCN-01 dosage was increased in Trial 2; UCN-01 low dose and high dose conditions were nM and 1250 nM, respectively. Cell proliferation was measured using an MTS cell proliferation assay compared against the control condition (was normalized to the cell proliferation = 100). Values were expressed as the mean $\pm$ standard deviation. Statistical significance was examined by analysis of a paired t-test, with $p < 0.05$ considered to indicate a statistically significant difference. **A.** High-dose (200 mM) duvelisib treatment was found to be more effective at reducing M41 cell viability in the older WI-38 fibroblast (p28) medium than young medium (p20, $p = 0.0004$). **B/C.** There is no statistically significant difference in M41's sensitivity to carboplatin when comparing the older and younger WI-38 (p20/21; p31/28) mediums at doses 20 μ M ($p = 0.0883$), 100 μ M ($p = 0.1043$, $p = 0.1387$), and 500 μ M ($p = 0.9314$). **C/D.** Initially found older WI-38 fibroblast (p28) coculture increases chemosensitivity for M41 OC cells treated with 250 nM UCN-01 ($p = 0.0016$), but subsequent experimentation revealed no statistically significant difference between older and younger medium conditions (p21; p28) at 250nM and 1250 nM UCN-01.

Impact of WI-38 Aging in Chemosensitivity with HGSOc-

Age being a significant risk factor for the development and progression of ovarian cancer, we wanted to investigate OC growth and chemosensitivity differences in “aged” versus “young” fibroblast coculture. The young fibroblasts

consisted of the youngest passage of W-38 human fibroblasts available, while the old fibroblasts were more advanced passages where slower growth was observed in culture but varies throughout the following experiments. The duvelisib treatment condition showed increased chemosensitivity in aged WI-38 (p28) cocultures (Figure 4A) compared with

the younger WI-38 cells (p20). Duvelisib low dose and high dose conditions were 40mM and 200mM, respectively. The higher duvelisib dosage of 200mM produced the greatest decrease in cell proliferation of M41 in the aged WI-38 coculture ($p = 0.0004$). In the first CRB trial (Figure 4B), the results showed no decreased cell proliferation when the cells were treated with CRB at high (100 μ M) or low (20 μ M) doses suggesting the drug doses were too low for M41 cells. We then increased the dosage of CRB from 20 μ M to 100 μ M for the low-dose condition and from 100 μ M to 500 μ M for the high-dose condition. In the second CRB trial (Figure 4C), appropriate decreases in cell proliferation were observed, but the differences in chemosensitivity between aged and young WI-38 conditions were not statistically significant (p -value = 0.5298). In this test, we also found the CRB doses in trial 2 were too high, killing most cells.

In the first UCN-01 trial (Figure 4D), we found a statistically significant reduction ($p = 0.0016$) in cell viability for M41 OC cells treated with 250 nM UCN-01 in the older WI-38 fibroblast (p28) coculture compared to the young WI-38 coculture (p21). The methodology was changed to increase the dosage of UCN-01 from 25 nM to 250 nM for the low-dose condition and from 250 nM to 1250 nM for the high-dose condition. In the second UCN-01 trial, no statistically significant decreases in cell proliferation

at 250nM and 1250 nM UCN-01 were observed (Figure 4E), and the differences in chemosensitivity between aged and young WI-38 conditions were not observed at both high and low chemotherapy doses.

HGSOCs HEYA8 and CAOV2 were cultured in WI-38 medium conditions for two consecutive 48-hours periods before being tested for relative cell proliferation using MTT assay. In the first trial, HEYA8 and CAOV2 were statistically significantly less proliferated (HEYA8: $p < 0.0001$, CAOV2: $p = 0.0367$) when cultured in aged WI-38 medium compared to young PDT conditions (Figure 5A). A second trial showed that HEYA8 was statistically significantly less proliferated ($p = 0.0047$) when cultured in 46-hour PDT WI-38 medium compared to 36-hour PDT conditions, but CAOV2 did not show statistically significant differences ($p = 0.2464$) between aged (PDT = 55) and young (PD = 34) medium conditions (Figure 5B). A third trial showed decreased HEYA8 and CAOV2 proliferation in aged medium conditions (Figure 5C), but the results were not statistically significant (HEYA8 $p = 0.6006$; CAOV2 $p = 0.4272$). OC cells were less proliferated when cultured in aged fibroblast (WI-38) medium in both HEYA8 and CAOV2 cell lines in all three replicate experiments, but only the first two trials had statistically significant results.

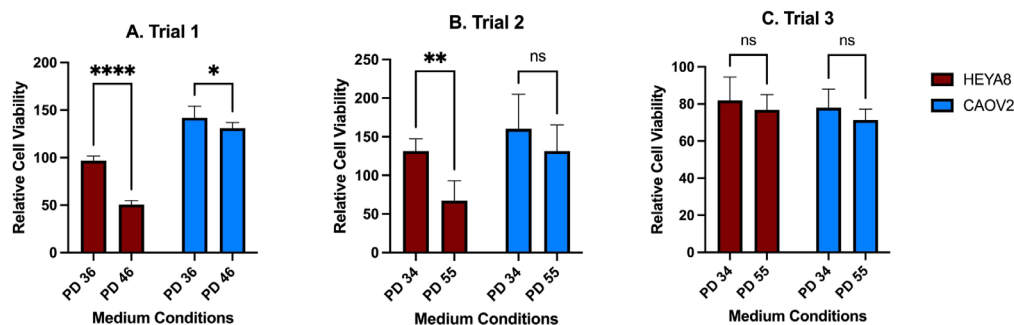


Figure 5. OC cells were less proliferated when cultured in aged fibroblast (WI-38) medium in both HEYA8 and CAOV2 cell lines. HGSOCs HEYA8 and CAOV2 were cultured in WI-38 medium conditions for two consecutive 48-hours periods before being tested for relative cell viability using MTT assay. **A.** HEYA8 and CAOV2 were statistically significantly less proliferated (HEYA8: $p < 0.0001$, CAOV2: $p = 0.0367$) when cultured in aged WI-38 medium with fibroblasts with a PDT of 46 hours compared to young, 36-hour PDT conditions. **B.** HEYA8 was statistically significantly less proliferated ($p = 0.0047$) when cultured in 46-hour PDT WI-38 medium compared to 36-hour PDT conditions. CAOV2 did not show statistically significant differences between aged and young medium conditions ($p = 0.2464$). **C.** A third trial showed decreased HEYA8 and CAOV2 proliferation in aged medium conditions, but the results were not statistically significant (HEYA8 $p = 0.6006$; CAOV2 $p = 0.4272$).

DISCUSSION

Chemosensitivity in OC Sub-types

In this study, we aimed to evaluate how sensitive various sub-types of OC would be to three different chemotherapies. Experiments were conducted that directly compared the effects of UCN-01, duvelisib, and ibrutinib on HGSOCS and CCOCs. As UCN-01,³¹ duvelisib, and ibrutinib target slow proliferating cells according to the previous findings from our lab, we expected OC cells with larger PDTs (slow cell proliferation) to be more sensitive to treatment. M41-cisR is a cell line generated from M41 with a cisplatin resistance. Comparison of M41 and M41-cisR cells showed increased ibrutinib sensitivity in cisplatin-resistant cells. Overall, slower proliferating cells, including cell lines OVCA432, M41, M41-cisR, OVCAR3, and PEO4, exhibited a statistically significant higher sensitivity to ibrutinib chemotherapy treatment compared to fast proliferating cells, which included the cell lines SKOV3, TOV21-G, and HEY. These results support our hypothesis and further investigation of ibrutinib as a therapy to target slower-proliferating cells, which are often the cause of OC recurrence as they can lie dormant in patients following initial chemotherapeutic treatment. Chemotherapies such as carboplatin and paclitaxel target fast-proliferating cells and will often fail to eliminate OC cells with higher PDTs, which is characteristic of slow proliferation.

In CCOCs, we did not see dose-dependent chemosensitivity to duvelisib or ibrutinib, but there was an increased sensitivity when compared to the HGSOCS control, HEYA8. CCOC cell lines JHOC5 and TOV21-G showed increased responses to higher doses of UCN-01, but OVISE showed little to no response when compared to the HEYA8 HGSOCS control. These results are surprising, as OVISE had the greatest PDT among the CCOCs, but showed the weakest response to UCN-01. This preliminary data suggested that the chemotherapeutic drugs, such as duvelisib, ibrutinib, and UCN-01, have differential responses among the subtypes of OC cells. Further tests will be performed to confirm this finding with the optimal drug doses.

Recent studies have found that BTK inhibitors

differentially suppress lipid accumulation in hematological malignancies,³² but there is very little data regarding solid cell tumors and lipid interactions in the context of BTK inhibitors. In terms of future directions to pursue regarding the data collected and presented in Figures 1, 2, and 3, looking at interactions between duvelisib and ibrutinib in varying lipid environments would be worthwhile. Initial data collected by our team suggests that high-fat lipid environments cause increased HGSOCS proliferation; the chemosensitivity test within a high-fat lipid environment has yet to be collected.

Many studies have shown ibrutinib's role as a platinum sensitizer,³³ suggesting the testing of ibrutinib as a polytherapy in multiple cisplatin-resistant OC cell lines should be of interest. Additionally, as OC is a cancer that often affects women in their 60s, studying differences in how duvelisib, ibrutinib, and UCN-01 interact in the aged versus young TME models—including aged lymphocytic environment as well as the other TME components—will be a future direction for this study, as we have yet to evaluate chemosensitivity in an aged versus young TME across sub-types and different OC cell lines.

Impact of Aged TME

In this experiment, we aimed to evaluate how the aged TME affects the proliferation and chemosensitivity of HGSOCS. Experiments were conducted that directly compared OC cell proliferation in an aged TME to the cell proliferation of OC found in the young TME. Then, chemosensitivity was evaluated in that aged and young TME. Previous in-lab study using rat OC xenograft models has shown increased tumor growth in aged rats when compared to young rats, which confirmed that age is a risk factor for OC development. Via RNAseq assay, we found that genes in tumor-surrounding tissues are differentially expressed between aged vs young rats. These results suggested that TME plays an important role in OC development. We therefore used aged WI-38 medium conditions to mimic the aged TME to test OC cell proliferation and chemo responses. Unexpectedly, our data showed that OC cells proliferated less when cultured in aged WI-38 medium in HEYA8 and CAOV2 cell lines (Figure 5). This seems

contradictory to our *in vivo* findings using rat OC xenograft models. This disagreement between the *in vivo* and *in vitro* studies was thought to be due to the single factors being tested *in vitro*, as the stromal fibroblasts WI-38 represent only one component of TME. In contrast, the *in vivo* environment is more complicated and includes multiple components, such as the immune system, blood vessels, ECM, etc. In the *in vivo* condition, the multiple components interact to regulate the cancer development and progress which is hard to achieve using *in vitro* models. The limitations have been reported by many studies that have shown the disagreement between *in vitro* and *in vivo* studies.³⁴

In Figure 5, HEYA8 OC cells were consistently less proliferative in the aged TME, but the results produced using CAOV2 cell lines were statistically significant in only Trial 1. Variabilities in CAOV2 results could be due to inconsistent techniques or cell numbers from WI-38 cells, as they grow more slowly as they reach senescence. In Trial 3, the use of a WI-38 cell line that had been in culture for a long period due to slow growth may be a possible reason for the insignificant differences between old and young environment-cultured HEYA8 and CAOV2. Additionally, in Trial 2, following the 4-hour incubation with MTT assay and the addition of isopropanol, the bottom of the clear wells looked ununiform in coloring, which could have resulted in data inconsistencies. Further research is required to investigate why OC was less proliferative in aged TME conditions, which is not aligned with the *in vivo* data using the rat xenograft OC model.

When the chemosensitivity was tested using OC and WI-38 fibroblast co-culture model, we found increased chemosensitivity in M41 OC cells treated with duvelisib but not carboplatin. The results were conflicting for UCN-01 chemotherapy conditions; only Trial 1 produced statistical significance, with M41 being less proliferative in the aged WI-38 environment high UCN-01 dose. We expected that aged TME, being closer to a senescent and slower proliferating state, would promote a more favorable response to drugs that more effectively target slow proliferating cancer cells rather than cells growing in the young TME, which would be more proliferative.

However, with statistical analysis of paired t-tests for all drug treatments, none of the results show a statistically significant difference currently (Figure 4). More experiments must be performed to further investigate the variable results seen in the CRB and UCN-01 chemotherapy treatments to rule out the possibility of technical issues contributing to the inconsistent data. Noticeably, the large standard deviation (SD) values for the CRB and UCN-01 treatments may indicate uneven cell numbers during transfer from transwell plates to 96-well plates. In Figure 4, we used WI-38 PDT 36 to represent the aged environment which may not be optimal, as higher PDT might be a better model for the test; this was implemented in the data from Figure 5. Theoretically, the larger the difference in PDT between young and aged WI-38, the more significant difference should be observed in cell proliferation between young and aged conditions of M41 culture. Higher accuracy in cell seeding and the changes in chemotherapy dosage would likely produce greater differences in the chemosensitivity of CRB and UCN-01 affected by the age of the TME as simulated by WI-38 cells, as well as more distinct cell proliferation differences in the absence of chemotherapy treatment.

In summary, we have found that slower proliferating OC cells exhibited a statistically significant higher sensitivity to chemotherapy treatment ibrutinib than faster-proliferating cells in HGSOCS. Cisplatin-resistant HGSOCS cell lines displayed increased chemosensitivity to ibrutinib compared with the cisplatin-resistant HGSOCS cell lines. In CCOCs, we did not see dose-dependent chemosensitivity to duvelisib or ibrutinib, but there was an increase in sensitivity when compared to the fast-proliferating HGSOCS control. CCOC cell lines JHOC5 and TOV21-G showed increased responses to higher doses of UCN-01, but OVISE, the slowest proliferating CCOC did not. It is of note that there are other morphological and genetic differences—beyond speed of proliferation—between the cell lines studied that warrant investigation.

When looking at OC cell proliferation in conditioned medium from aged versus young WI-38 fibroblasts, OC cells were less proliferative in aged WI-38 medium when compared with the OC cells in young

WI-38 medium in both HEYA8 and CAO V2 cell lines. Furthermore, we found that older WI-38 fibroblast coculture increased chemosensitivity for M41 OC cells treated with duvelisib but not carboplatin; the results were inconclusive for UCN-01 chemotherapy conditions. Future studies will be focused on the mechanisms of TME in cancer cell growth and chemo-responses, utilizing aged and young lymphocytes in addition to the WI-38 fibroblasts. Future directions could also include differentiation of preadipocytes in order to manipulate lipid content in medium, so that we may better understand lipid effects on OC proliferation and chemosensitivity, as well as how BTK inhibitors differentially suppress lipid accumulation in a setting catered to solid tumors.

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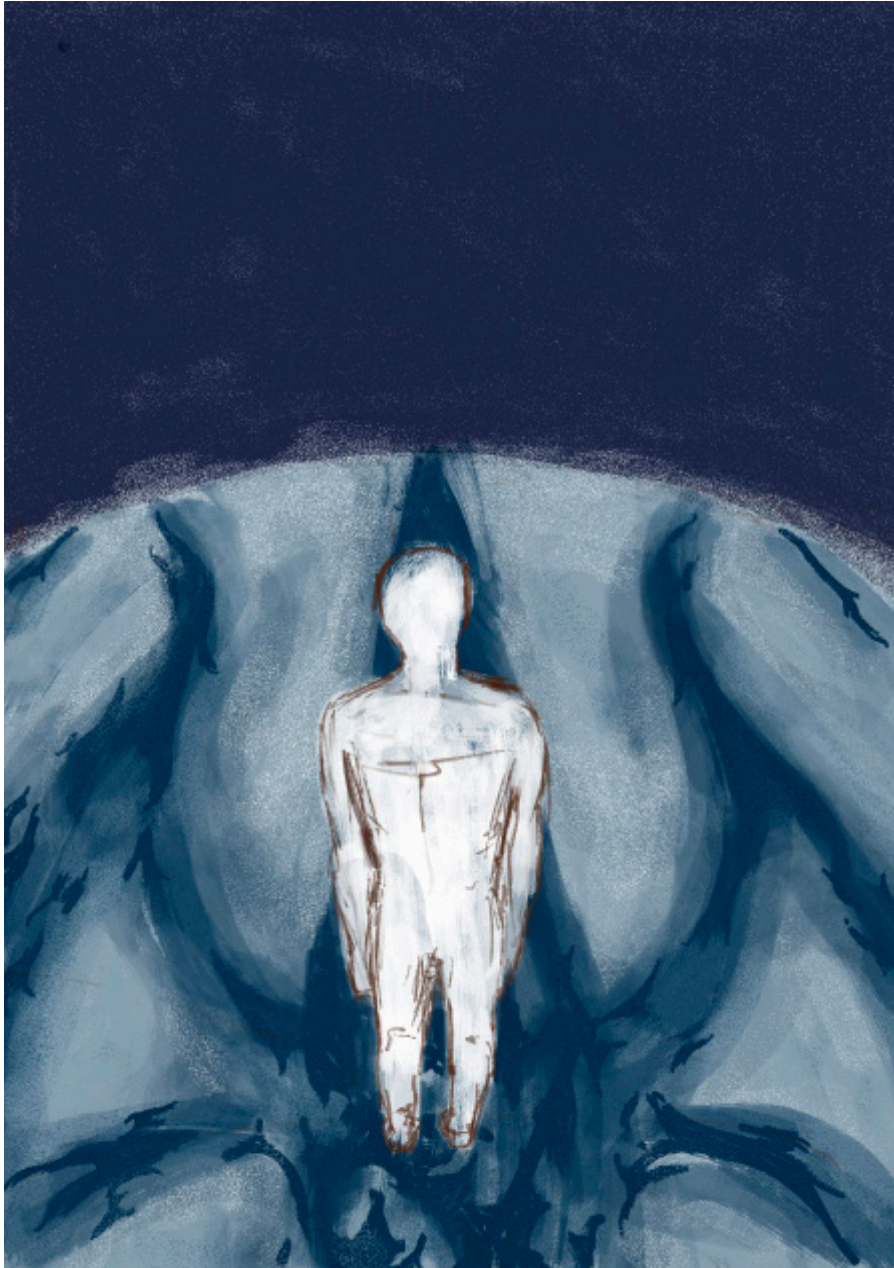
Identity, Resilience and their Psychological Impact Among Older Adults with Cancer

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Article Synopsis

This paper investigates the psychological impact of identity and resilience on the distress experienced by older adults with cancer. To do this, we synthesized the literature surrounding this topic from the past two years (2022-2024). Findings indicate that mental health interventions such as Self-System Therapy (SST) can improve distress and address support needs for older adults with cancer.



Graphic by Monet Shum

Identity, Resilience and their Psychological Impact Among Older Adults with Cancer

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ABSTRACT

Older adults (65+) make up 60% of cancer diagnoses and 70% of deaths related to cancer (Marosi & Köller, 2016). Although this age group experiences significant distress, behavioral studies investigating distress tend to be overrepresented by younger populations, leaving a gap in the knowledge about the distress needs of older adults (Chang et al., 2020). To bridge this gap, Dr. Katherine Ramos and Dr. Timothy Strauman of Duke University's Psychology Department conducted a pilot study that found two major themes related to distress in older adults: identity and resilience. The purpose of this study is to investigate the psychological impact of identity and resilience on the distress experienced by older adults with cancer. Using the two qualitative themes, identity and resilience, the researchers of this study conducted a narrative synthesis of contemporary literature surrounding identity, resilience, and their psychological impact on distress in older adults with cancer. Following the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines, the study found nine eligible articles. The study conducted a thematic analysis of the articles in this synthesis and found six themes: (1) reconstructing identity after diagnosis, (2) experiencing anxiety due to uncertainty of cancer outcomes, (3) needing to "push along" through a cancer diagnosis, (4) having access to social support increases resilience, (5) experiencing low resilience leads to demoralization, and (6) navigating cancer requires redefining oneself to confront cancer challenges. Future directions and clinical implications include adapting Self-System Therapy (SST) as a mental health intervention for older adults with cancer.

INTRODUCTION

Cancer remains a significant global health challenge and is one of the leading causes of death worldwide (Roser & Ritchie, 2019). Its impact is far-reaching and complex, affecting individuals, families, and healthcare systems. The incidence of cancer is particularly notable among older adults, specifically those aged 65 and older. This demographic accounts for approximately 60% of all cancer diagnoses. Moreover, about 70% of deaths caused by cancer occur in this age group (Marosi & Köller, 2016). This highlights a critical need for targeted research and intervention strategies.

Despite the high prevalence of distress among older adults, a notable gap exists in behavioral studies focusing on distress in older adults. Much of the existing research on distress is primarily

concentrated on younger populations, leaving a void in understanding the specific needs and experiences of older adults (Chang et al., 2020). This oversight in the literature underscores the urgency for more age-specific studies that address the unique challenges faced by older adults.

To address this gap, Dr. Katherine Ramos and Dr. Timothy Strauman conducted a pilot study to understand the specific distress needs and sources of support for older adults with advanced lung cancer. Data from this study found five key themes: uncertainty, managing symptoms and side effects, identity, finding support, and resilience (Ramos & Strauman, 2022). This paper will concentrate on two of these themes: identity and resilience. Ramos & Strauman (2022) found that older adults with advanced lung cancer experience a significant

loss of identity after diagnosis. They also found that resilience emerges from a multitude of factors such as the support systems that the patient leans on and the active choice the patient makes to focus on the positive and live in the present moment. The purposes of this thesis are to examine these themes across cancer types more broadly, exploring their connections to distress among older adults with cancer. This study hopes to drive more mental-health oriented and comprehensive cancer supportive care in older adults.

Identity, or how one and others perceive themselves, has been profoundly affected in older cancer patients (Bilgrami, 2006). Many older adults with cancer expressed a shift in their self-view after being diagnosed with cancer, transitioning from seeing themselves as healthy individuals to identifying as patients grappling with a severe illness (Ramos & Strauman, 2022). This altered sense of self significantly influences their overall distress. Distress tends to increase as one becomes an older adult through factors such as a lower sense of control, widowhood, and declining levels of health. Cancer diagnosis exacerbates this increasing distress (Schieman et al., 2001). Understanding the relationship between identity and cancer distress in older adults can improve cancer care and reduce distress for this vulnerable population.

Another aspect of an older adult's altered sense of self due to their cancer diagnosis is how other's perception of them changes. Many older adults communicated that keeping their diagnosis private is very important, with only close family and friends informed about ongoing developments. They did not want others in their community to view them as a cancer patient or to be treated differently due to their diagnosis (Ramos & Strauman, 2022). As an individual diagnosed with cancer, the altered sense of self is reinforced by others unintentionally treating them like a dependent or unhealthy person.

Resilience, defined as the capacity to maintain stability in the face of adversity, is often misconstrued as an innate trait (Southwick et al. 2014). However, it is more accurately shaped by one's experiences, environment, and the quality of support systems,

including financial resources, family, educational institutions, and their broader community (Pooley & Cohen, 2010). Support systems and facilitative environments can even prevent negative consequences of traumatic experiences by promoting positive growth, such as successful adaptation and coping to stress, following exposure to traumatic events (Ungar, 2013). If an individual with a cancer diagnosis lacks many of these support systems, their resiliency will most likely be low which may correlate with greater mental and physical burdens and subsequently exacerbate their distress (Sihvola et al. 2022).

By reviewing this literature from the past two years, this study creates a narrative analysis of distress experienced by older adults with cancer captured under the lenses of identity and resilience. This review puts forth the current knowledge on this topic to allow future researchers to understand what questions to explore. By focusing on these themes, this research hopes to contribute valuable insights to the field of oncology and gerontology, ultimately informing more effective and empathetic approaches to supportive cancer care for this vulnerable population.

METHODS

Selection Criteria

The inclusion and exclusion criteria for the review are depicted in the table below.

Table 1.

Inclusion Criteria	Exclusion Criteria
Participant age ≥ 50 years	Participant age < 50 years
Participant has cancer (any stage; disease agnostic)	Any other chronic disease
Outcomes related to resilience, identity, and self-concept	No focus on cancer patient
Qualitative, quantitative, behavioral trials, and supportive care interventions	Pharmacological, clinical (e.g., drug trials) or lab-based studies

Methodology

Referencing the eligibility criteria above, articles were searched for using the databases APA PsycArticles and APA PsycInfo under the EBSCOhost platform to conduct the initial three searches. For each search, the advanced search option was used to mark the options of peer-reviewed papers, age groups of aged (65 years and older) and very old (85 years and older), and a date range of 2022 to 2024. The first search included the terms “cancer” and “identity” yielding 27 articles sorted into the identity batch. The second search included the terms “cancer” and “resilience or resiliency or resilient” yielding 37 articles sorted into the resilience batch. The third search included the terms “cancer” and “self-esteem or self-concept or self-worth or self-evaluation or self-perception” yielding 27 articles sorted into the self-concept batch.

Next, the duplicates were eliminated from each batch of articles, removing 1 article from the identity batch, 1 article from the resilience batch, and 4 articles from the self-concept batch. After this, the first review of all the articles was conducted using the exclusion criteria. This resulted in removing 12 articles from the identity batch, 19 articles from the resilience batch, and 8 articles from the self-concept batch. Then, the second review of the articles was conducted using the inclusion criteria. This resulted in removing 4 articles from the identity batch, 13 articles from the resilience batch, and 12 articles from the self-concept batch.

After sorting through the articles using the inclusion and exclusion criteria, there were 10 articles in the identity batch, 4 articles in the resilience batch, and 3 articles in the self-concept batch. Next, an abstract review of each of the articles was conducted that sorted out articles that did not meet our relevant outcomes of identity, resilience, or self-concept. In this review the abstract and methods section were read. This resulted in 6 articles in the identity batch, 4 articles in the resilience batch, and 1 article in the self-concept batch. Finally, a full-text review was conducted that sorted out articles that did not meet the relevant outcomes. In this review, each article was read in full. This resulted in the final collection of 5 articles in the identity batch, 3 articles in the resilience batch, and 1 article in the self-concept batch

with a total of 9 articles in our review. See Table 5.

With the final articles selected, a thematic content analysis was conducted to find the most recurring patterns and themes within the articles under the identified outcomes. Under the identity outcome three themes were found. Under the resilience outcome two themes were found. Under the self-concept outcome one theme was found. Through these themes a narrative analysis was created that demonstrates how these themes relate to their respective outcomes. See Figure 1. Consort Diagram.

Table 5. *Articles in final batch*

Identity Articles	Population of Study	Study Design	Variables/Outcomes
Ciaralli et al. 2021 *age range from 58-95	-older adult cancer survivors -60 years +	-data from older study -statistical analyses -differences between means -bivariate correlation analysis -three ordinary least-squares regression analyses -regression analyses	-perceived disability (1 item) -demographic and personal characteristics -# of comorbidities -level of reported functional difficulties (nagi 1976 index) - # of current non-cancer symptoms -# of treatment types -cancer stage -current cancer symptoms
Harmon and Kyle 2019	-cancer/ remission patients -M=67 yrs, Range: 58 yrs -73 yrs	-semi-structured interviews based upon prior informal discussion	-historical relationship to nature and leisure -diagnosis of cancer and accompanying treatment program -participation in hiking program -outlook for future
Martin et al. 2023 *note that two participants were in 40s	-those who had completed treatment for bowel cancer -M=63 yrs, Range: 42 yrs – 92 yrs	-interpretive descriptive qualitative methodology -inductive analytic approach	Themes -I had to heal myself -Life's too short -The person I've become
van der Kamp et al. 2022 *one participant is 47	-men w/ prostate cancer -M=62 yrs, Range: 47 yrs – 71 yrs	-narrative analysis	Themes -reconstructing identity -shifting relationship w/ prostate cancer -relationship w/ technology -uncertainty, incoherence and haunting

Yeung et al. 2022 Mean age is 68.75 SD=10.66	-rural patients w/ advanced cancer -M = 68.75 yrs; SD = 10.66	-qualitative descriptive design -semi-structured interviews w open ended questions	-demographic info -clinical and disease info Themes -fluidity in patients' emotions -religion/spirituality -social support -positive attitude -cognitive strategies -self-care -medication
Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Chen et al. 2021	-Cancer patients in Taiwan -M=67.18 yrs; SD=10.82; Range = 42 yrs – 87 yrs	-Advanced Care Plan	-coping scale ways of coping checklist-revised -social support scale -resilience scale
Chien et al. 2022	-Prostate cancer survivors - age range from 46-84, median=69 and mean=68.82, SD=6.74	-cross-sectional -self reported questionnaires	-disease characteristics -physical symptoms (mandarin version of expanded prostate cancer index composite) -cancer specific self-efficacy (mandarin version of cancer survivors' self-efficacy scale) -psychological resilience (mandarin version of connor-davidson resilience scale) -demoralization (mandarin version of the demoralization scale)

Michael et al. 2022	-Cancer patients - age range 44-99, mean=71.8, SD=11.4	-video intervention vs standard -quantitative and qualitative	Themes -cancer patients ability to posture vulnerability w/ resilience Subthemes -acknowledging frailty w/ diverse adaptations to dependency -benevolence and reciprocation of family/friends caring -death anxiety, ambivalence and experiential acceptance of dying -honouring death through secular and sacred rituals
Self-Concept Articles	Population of Study	Study Design	Variables/Outcomes
Törnävä et al., 2021	Men w penile cancer surgery -age range 50-91	Interview or response letter -thematic analysis	Themes -cancer-modified me -everyday life defined by physical symptoms -sexual life defined by cancer -reshaped content of life

RESULTS

Under the following sections, the literature review identified nine articles that fit the parameters of the search across identity (n=5), resilience (n=3), and self-concept (n=1).

Identity

Identity is defined as how an individual and others perceive themselves (Bilgrami, 2006). Cancer and its treatment affect a patient's identity by altering their sense of self, shifting their identity from a healthy, independent self to an unhealthy, dependent self. Others around them, such as family and community members, also start treating them differently, bringing "cancer patient" to the forefront of their identity (Ramos & Strauman, 2022).

Within this first batch of five articles, studies primarily focused on how cancer affects the lives of older adults and the general experiences of being an individual with a cancer diagnosis. These studies

used qualitative research designs that incorporated interviews. See Table 5 for more study details. After reviewing these five articles, three themes emerged: reconstructing identity after diagnosis, experiencing anxiety due to uncertainty of cancer outcomes, and needing to "push along" through a cancer diagnosis.

Reconstructing Identity After Diagnosis

Patients expressed that much of their current identities are lost and replaced with a "patient" identity (van der Kamp et al., 2022). Patients had different ways of reconstructing their identity to cope with this lost sense of self. Some patients wanted to suppress the "patient" identity by acting strong, independent, and resilient. These patients would hide their emotions and suppress their fears: "[My daughter] used to say to me, 'how are you feeling?' and I used to think 'I can't be bothered talking about feelings'" (Martin et al., 2023). This resulted in patients becoming more irritable, angry, and less empathetic. Patients cited annoyance at other's worries as they were perceived

as minor compared to their own experiences with cancer. Some of these patients communicated that they preferred their pre-diagnosis identity, with one participant admitting “she ‘*rather liked the person [she] was before*’” (Martin et al., 2023).

Other patients reestablished their sense of self by reconnecting with identities from their past. Patients communicated that as they aged and life became more monotonous and repetitive, they drifted away from who they used to be. However, being diagnosed with cancer disrupted the tediousness of life with intense negativity and a loss of self. This disruption allowed patients to reconsider what was important to their life and identity: “Cancer was a real wakeup call. ‘*Everything that was really important to me came back front and center*’” (Harmon & Kyle, 2022).

Patients came back, within the limitations of their current health, to activities, friendships, and anything else that used to give their lives value and meaning. Even with these imposed limitations, these past identities incurred feelings of nostalgia and a newfound appreciation for life (Harmon & Kyle, 2022; van der Kamp et al., 2022). A diagnosis of cancer takes away the implicit and unappreciated value of life by creating immense instability in its continuity. To regain a meaning of life, patients returned to former identities that shaped their core self and interlaced it with their “patient” identity to create a new intermingled self. van der Kamp et al. (2022) corroborates this finding: “We saw that reconstructing identity consists of memoirists referring to previous identities and connecting them to their current identity of being a [...] cancer patient”.

Experiencing Anxiety Due to Uncertainty of Cancer Outcomes

Cancer patients experience anxiety due to the uncertainty of cancer outcomes. This uncertainty comes from various reasons. Some patients’ uncertainty stemmed from discrepancies between their subjective sense of health and their objective health. Patients did not always feel bad, citing that sometimes they would feel very good: “‘*Because I feel good. So good that I sometimes wonder if I really have cancer*’” (van der Kamp et al., 2022). The contradiction in identity, between the mind and body,

causes anxiety and feelings of uncertainty. Patient’s uncertainty also stemmed from incongruency between multiple doctors’ opinions and even between these opinions and test results. Patients worry about their current state of cancer, if the doctors and tests are accurate, and if their cancer will spread and worsen in the future. One participant wrote, “‘*Realistically there was a myriad of what ifs lurking down the road, most beyond my control, to be resolved by the sometimes unsympathetic roll-of-the-dice. What if the tests were wrong and the cancer had actually spread? What if I made the wrong decision as to how I was to be treated?*’” (van der Kamp et al., 2022). This ongoing anxiety of current and future health leaves the patient in a continuous cycle of worry (van der Kamp et al., 2022).

For patients in remission, there is always a worry of cancer recurrence. This constant anxiety makes patients hypervigilant to their body functioning. Slight aches and pains would trigger immense anxiety and concern of relapse. Multiple participants exhibited this fear: “Even a minor ‘*little niggle*’ (Julie) could prompt a rapid and intense anxiety response with the overwhelming thought ‘*Oh my god, is it back?*’ (June)” (Martin et al., 2023). Patients referred to this constant worry as existential angst, making previously inconsequential matters drive feelings of anxiety. This anxiety became an unwelcome part of their new identity, with patients lacking confidence, a trait which they previously identified with having (Martin et al., 2023).

Needing to “Push Along” Through a Cancer Diagnosis

Patients discussed feeling the need to “push along” and keep fighting to survive through their cancer diagnosis. This feeling arose from a variety of reasons. Some patients wanted to identify as strong and resilient so they would “get on” with life, doing the best that they could. One patient stated “‘*I think it’s just the attitude. I just gotta keep pushin’ myself and keep goin’*. If I sit in the chair, that’s gonna be the end’” (Yeung et al., 2022). Many patients had internally driven expectations to remain positive by expressing their strength and resilience (Yeung et al., 2022). These expectations of remaining strong,

resilient, and positive can also be externally driven. External pressures include viewpoints of what makes a cancer survivor. Patients describe the constant advice from others to “remain positive” and “stay strong” in the face of cancer (Martin et al., 2023). If a patient could not adhere to this, it was a visual representation of the cancer winning. This pressure to remain positive and strong led patients to hide their true feelings. One anecdotal quote includes: “*I’m pretty good at being able to put the face on and just go and do it, and then on the inside be falling to pieces, and sit in the car and have a really good cry*” (Martin et al., 2023). While remaining positive and strong allows patients to “push along” through a cancer diagnosis, it also causes patients to mask their true feelings.

Patients in remission felt like they must “push along” due to the opportunity of life they had been given. Many expressed that others in their situation are dead and they are the lucky ones that are alive. Even if they have had many resulting complications, the patients communicated that it was better than being dead. One participant stated, “*It could be better, but it could be a lot worse too, eh. I could be dead*” (Martin et al., 2023). They felt like they were wasting this second chance at life if they did not “push along” (Martin et al., 2023).

Resilience

Resilience is defined as the capacity to maintain stability in the face of adversity (Southwick et al., 2014). Although resiliency is typically misunderstood as an innate trait, it is indeed shaped by one’s experiences, environment, and the quality of support systems, including financial resources, family, educational institutions, and their broader community (Pooley & Cohen, 2010).

Within this second batch of three articles, studies primarily focused on the various factors that influence a cancer patient’s resilience across both qualitative and quantitative research designs, primarily using self-reported questionnaires. See Table 5 for more study details. After reviewing these three articles, two themes emerged: having access to social support increases resilience and experiencing low resilience leads to demoralization.

Having Access to Social Support Increases Resilience

Overall, it was found that social support is significantly and positively correlated with higher use of problem-focusing coping strategies, which increases a patient’s resilience because they can manage their distress on their own. Problem-focused coping is defined as an individual intentionally deciding to manage the source of stress in a troubling situation. Cancer patients with higher levels of social support made more use of problem-focused coping strategies, such as modifying a situation to make it less stressful, to manage the distress they experience due to their cancer diagnosis (Chen et al., 2021). Previous research found that patients with higher resilience display better active coping strategies, such as positive reappraisal and planful problem-solving (Lee & Kim, 2018; Popa-Velea et al., 2017). Since higher social support correlates with greater use of coping strategies and greater use of coping strategies is associated with higher resilience, it can be inferred that higher social support is correlated with higher resilience. Overcoming adversity is also a part of the experiences that shape an individual’s resilience. Therefore, having concrete strategies like problem-focused coping allows cancer patients to overcome adversities related to their cancer diagnosis and increase their resilience.

Cancer patients also rely on social support for both physical and emotional needs. Many of the patients identified how their loved ones will manage household tasks such as cooking and cleaning, transportation, and even personal hygiene. When discussing this topic, one participant explained that “*physical tasks like cleaning and gardening can be very difficult or impossible, but my family looks after me*” (Michael et al., 2022). Patients also stated that spending time with family and friends allowed them to live a meaningful life. The care given by their loved ones produced a sense of being valued. Michael et al. (2022) affirmed this sentiment: “The reciprocation of care experienced within the patient-caregiver dyad engendered much hope for many. Rather than experience a state of dejection, caregivers’ emotional and moral solidarity installed a sense of security”. Patients also expressed their

indispensable reliance on their family and friends (Michael et al., 2022). The outcome of feeling valued from this care encompasses the experiences that shape one's resiliency, illustrating that social support is associated with resilience.

Experiencing Low Resilience Leads to Demoralization

Demoralization is the psychological state in which an individual feels hopelessness, despair, helplessness, meaninglessness, and a deprivation of goals (Chien et al., 2022). Chien et al. (2022) found "that survivors with higher psychological resilience experienced less demoralization", demonstrating that focusing on improving a patient's psychological resilience will reduce demoralization. Cancer-specific self-efficacy, the confidence an individual has in their abilities to self-manage health problems related to cancer and its treatment, was found as a protective factor for both psychological resilience and demoralization (Chien et al., 2022). Therefore, focusing on improving a patient's cancer-specific self-efficacy will improve psychological resilience, thus also reducing their demoralization. Improving cancer-specific self-efficacy would likely include teaching coping strategies and increasing access to resources (medication, tools, technology) that allow the patient to self-manage health problems (Chen et al., 2021; Chien et al., 2022). These coping strategies and resources encompass the support systems that shape an individual's resilience, demonstrating how focusing on improving cancer-specific self-efficacy would heighten a cancer patient's resilience and hence lower their levels of demoralization.

Self-Concept

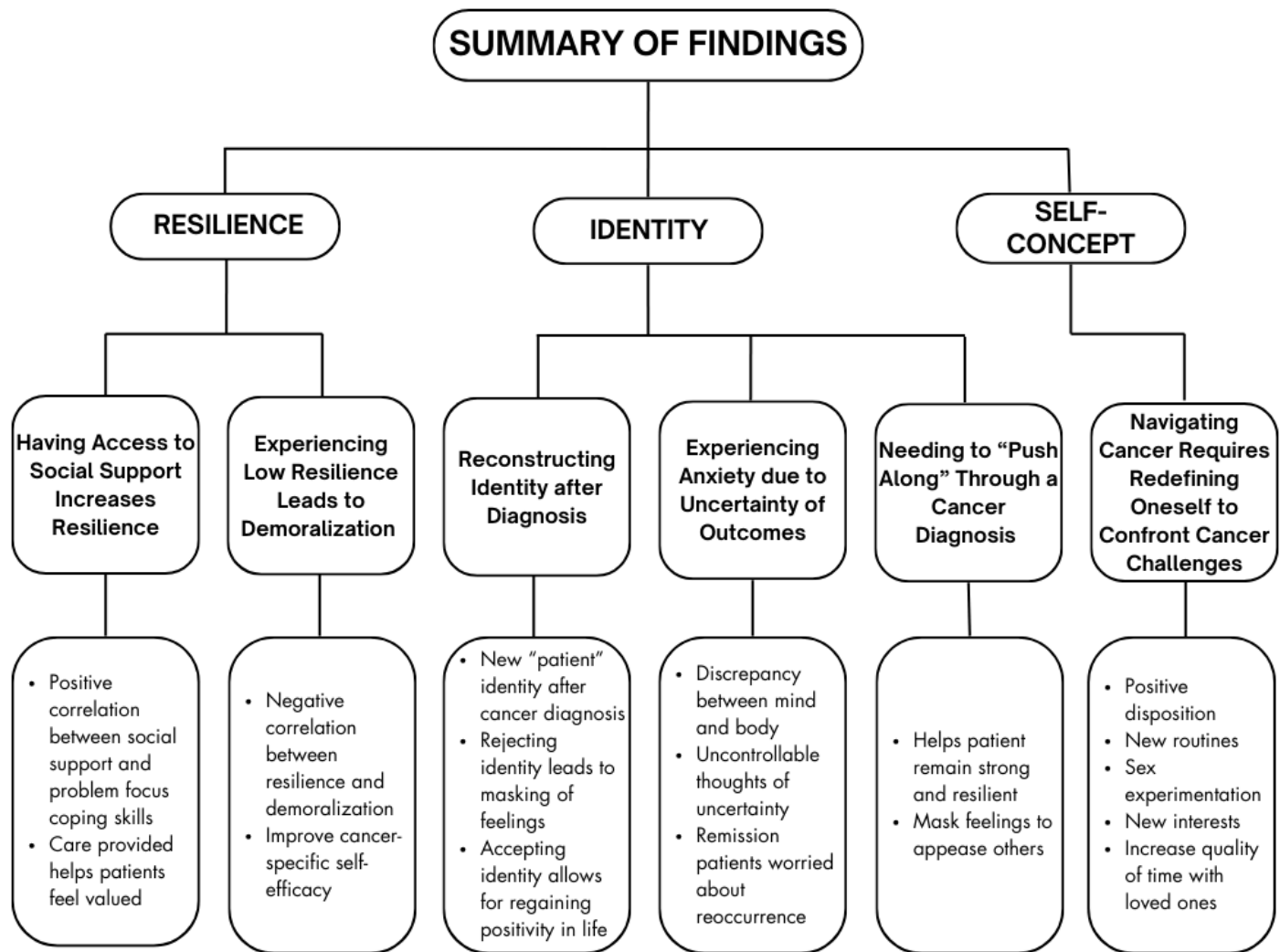
While identity includes how others perceive an individual, self-concept is focused on how the individual cognitively perceives themselves (Kinch, 1963). Cancer and its treatment affect how a patient conceptualizes themselves, typically leading to a negative view of oneself based upon their limited autonomy and worsened health (Bhattacharjee, 2013).

Within this third batch there was one article, which focused on the various aspects of life impacted by cancer and how older adults adapted to these

changes. The study design was qualitative using interviews to collect information. See Table 5 for more study details. After reviewing this article, one theme emerged: navigating cancer requires redefining oneself to confront cancer challenges.

Navigating Cancer Requires Redefining Oneself to Confront Cancer Challenges

Participants described many ways in which their lives have permanently changed after diagnosis and surgery. Even though the participants struggled with many challenges related to cancer, the participants were able to cope with new lifestyle changes by redefining their self-perception. To combat negative feelings such as shame, inadequacy, and stress, participants became more positive, vulnerable, humorous, and joyous. One patient expressed, "*I have tried with light humour to replace difficulties; things were going forward when you look from a different perspective and look for humour in different events. That is the way to make life easier*" (Törnävä et al., 2022). To cope with limited physicality due to daily pain, participants developed daily routines that allowed them to maintain their physical functioning. One participant articulated, "*I cannot work as before, but I can take care of myself, cook, go shopping. I can go in for sports, go for small walks, ride a bike*" (Törnävä et al., 2022). To adjust to a new sex life, participants were open to experimentation and redefined what was important to them in sex. One patient communicated, "*Well, you can find all kinds of sex tools like strap-ons and whatever you can find now. Those have been good and everything else and, yes, we have always been like this with my wife*" (Törnävä et al., 2022). To adjust to a new life, participants engaged in new interests that fit into their physical limitations and spent more time with family to increase meaning and joy in life. Törnävä et al. (2022) expressed the following: "Children and grandchildren were perceived as a mental resource that gives meaning to life and increases the joy of life". Although these participants dealt with many hardships due to their diagnosis, they positively adapted themselves to be able to confront these challenges and live a meaningful life (Törnävä et al., 2022).



DISCUSSION

The aim of this narrative synthesis was to review the current literature on older adults with cancer and determine the psychological impact of identity and resilience on their distress. This review was able to highlight and emphasize these psychological impacts through three relevant outcomes: identity, resilience, and self-concept. Through a thematic content analysis, this study proposed 3 themes under identity, 2 themes under resilience, and 1 theme under self-concept.

The three themes found under identity were reconstructing identity after diagnosis, experiencing anxiety due to uncertainty of cancer outcomes, and needing to "push along" through a cancer diagnosis. Older adults expressed that after diagnosis, all their previous identities vanished and were replaced with

"patient". They either rejected this identity by acting strong, hiding their emotions, and suppressing their fears or accepted this identity by interweaving it with former valuable identities and lifestyles. The former became irritable and less empathetic, while the latter regained a meaning of life. This finding is corroborated in Szcześniak et al. (2020), which found that disease acceptance is positively correlated with meaning in life. Participants' anxiety of uncertainty was due to a contradiction between their subjective and objective health, lack of consensus between doctors and test results, and cancer recurrence, if in remission. Anxiety became an unwelcome facet of their post-diagnosis identity, contributing to the existing literature on the correlation between uncertainty and anxiety in older adults with cancer (Verduzco-Aguirre et al., 2020). Participants discussed the need to "push along" to get through cancer. However, for some this was an external

pressure that caused them to mask their true feelings. Rosedale (2009) had a similar finding in which breast cancer survivors withheld their cancer experiences and masked their feelings to appear resilient and strong.

The two themes found under resilience were having access to social support increases resilience and experiencing low resilience leads to demoralization. Higher levels of social support were associated with higher use of problem-focused coping which can help patients overcome adversities related to their cancer. Overcoming adversity is an important aspect of resilience, demonstrating, in accordance with previous literature, how access to social support is associated with increased resilience (Hu et al., 2018; Somasundaram & Devamani, 2016; Vartak, 2015). Having low resilience can lead to demoralization in cancer patients. To increase resilience, and therefore lower demoralization, improving cancer-specific self-efficacy should be focused on as this is a protective factor for both. Liu et al. (2022) had similar findings in which they found that kidney cancer patients with better cancer-related self-efficacy had greater resilience.

The theme found under self-concept is navigating cancer requires redefining oneself to confront challenges. Older adults with cancer had to make changes to *the self* to endure the challenges of cancer, such as becoming more vulnerable and positive, changing interests, and adjusting their allocation of time. By accepting and redefining oneself considering their diagnosis, they can navigate the obstacles of cancer while bringing positive change to their life. Previous research supported this finding such as Connerty and Knott (2013), which found that making lifestyle changes allowed cancer patients to experience positive change and growth in their lives.

These findings allow us to understand how identity and resilience psychologically impact older adults with cancer and the best practices to improve distress and mental health for these individuals. Older adults with cancer can regain meaning and an appreciation for life by accepting their "patient" identity and combining it with their past identities. Healthcare providers should be aware of and address the various

uncertainties surrounding cancer and its treatment to reduce anxiety in cancer patients. Cancer patients need to balance their will to "push along" with their need to express their true feelings and emotions. This will allow them to remain strong and resilient while still being able to express their vulnerability. Cancer patients stand to benefit from leaning on their family, friends, and community as high levels of social support are correlated with higher resilience. Cancer patients could also benefit from being taught coping strategies and given access to resources (medication, tools, technology) to improve their ability to self-manage problems relating to cancer and its treatment (cancer-specific self-efficacy) as this is a protective factor against demoralization and improves resilience. Finally, to be able to confront the challenges related to a cancer diagnosis, patients may want to find opportunities to redefine themselves in many aspects of life, such as becoming more positive and humorous, developing or changing daily routines, engaging in new interests, and spending more quality time with loved ones. Patients will experience these changes as positive, and it will allow them to live a meaningful life.

Strengths and Limitations

While we tried to be comprehensive in our study, a formal meta-analysis could not be conducted due to the low number of eligible articles in our study. Given this low number, the diversity across study samples was also somewhat limited. We opted for a narrative synthesis instead, reviewing research articles from the past two years. By conducting a narrative synthesis within two years, we ensure our research is utilizing the most relevant information for our research question, particularly when a full meta-analysis is beyond the scope of our aims. Moreover, this approach allows us to identify emerging trends, summarize existing evidence, and provide practical insights to inform future research. The qualitative nature of our synthesis allows for more personalized and humanized narratives of living with cancer as an older adult. Some of the eligible studies had participants between the ages of 40 and 50, which deviates from the inclusion and exclusion criteria. However, we opted to include these studies because participants within 1 to 2 standard deviations away

from the mean ages were still within our age range of 50 years and older. Another limitation is the narrow breadth of our search, only using two databases and limited search terms. Even with this small scope, this review illustrates the most contemporary literature on this topic, giving researchers the most current knowledge to base their future studies.

Future Directions and Clinical Implications

Future research should focus on mental health interventions for older adults with cancer. While current research has identified mental health issues and what can lead to their improvement in older adults with cancer, mental health remains largely untreated among this older adult population, despite how common these problems are for this demographic (Carlson et al., 2004). Interventions should focus on how to implement the above findings on distress and mental health in older adults with cancer. Social support greatly increases resilience, but how do we increase social support for this population? Creating a new intermingled identity helps patients find meaning and value in life, but how do we facilitate this reconstruction of identity? "Pushing along" through a cancer diagnosis allows patients to remain strong and resilient, but it can cause patients to mask their true feelings. So, how do we both allow patients to express themselves while not allowing them to feel weak and helpless?

To bridge this gap, Dr. Ramos and Dr. Strauman developed a behavioral intervention tailored for older adults with advanced lung cancer. This behavioral intervention used Self- System Therapy (SST) to address the distress needs of this patient population. SST is an evidence-based psychotherapy originally used to treat depression that is grounded in Higgins' regulatory focus theory (Veith et al., 2003). This theory defines self-regulation as a distinction between a prevention focus, avoiding pain, and a promotion focus, approaching pleasure (Higgins, 1977). SST states that depression can be improved by moving away from a prevention based motivational system, which centers on loss aversion and fulfilling responsibilities to avoid negative outcomes, and towards a promotion based motivational system, which focuses on advancing towards goals and

emphasizes gains. While SST was adapted for older adults with advanced lung cancer, this intervention could be more broadly adapted for older adults with any type of cancer to facilitate better mental health outcomes for this vulnerable population.

In conclusion, targeted interventions such as SST, which emphasize resilience and identity, show promising potential for significantly supportive care of older adults with cancer. The diverse needs of this demographic underscore the necessity for treatment approaches that are both flexible and inclusive. Moreover, involving care partners represents a valuable future opportunity to advance the field of supportive care in oncology and gerontology. By adopting these holistic strategies, we can enhance the well-being and quality of life for older cancer patients, ensuring that care is not only effective but also compassionate and comprehensive.

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APPENDIX

Figure 1. *Consort Diagram*

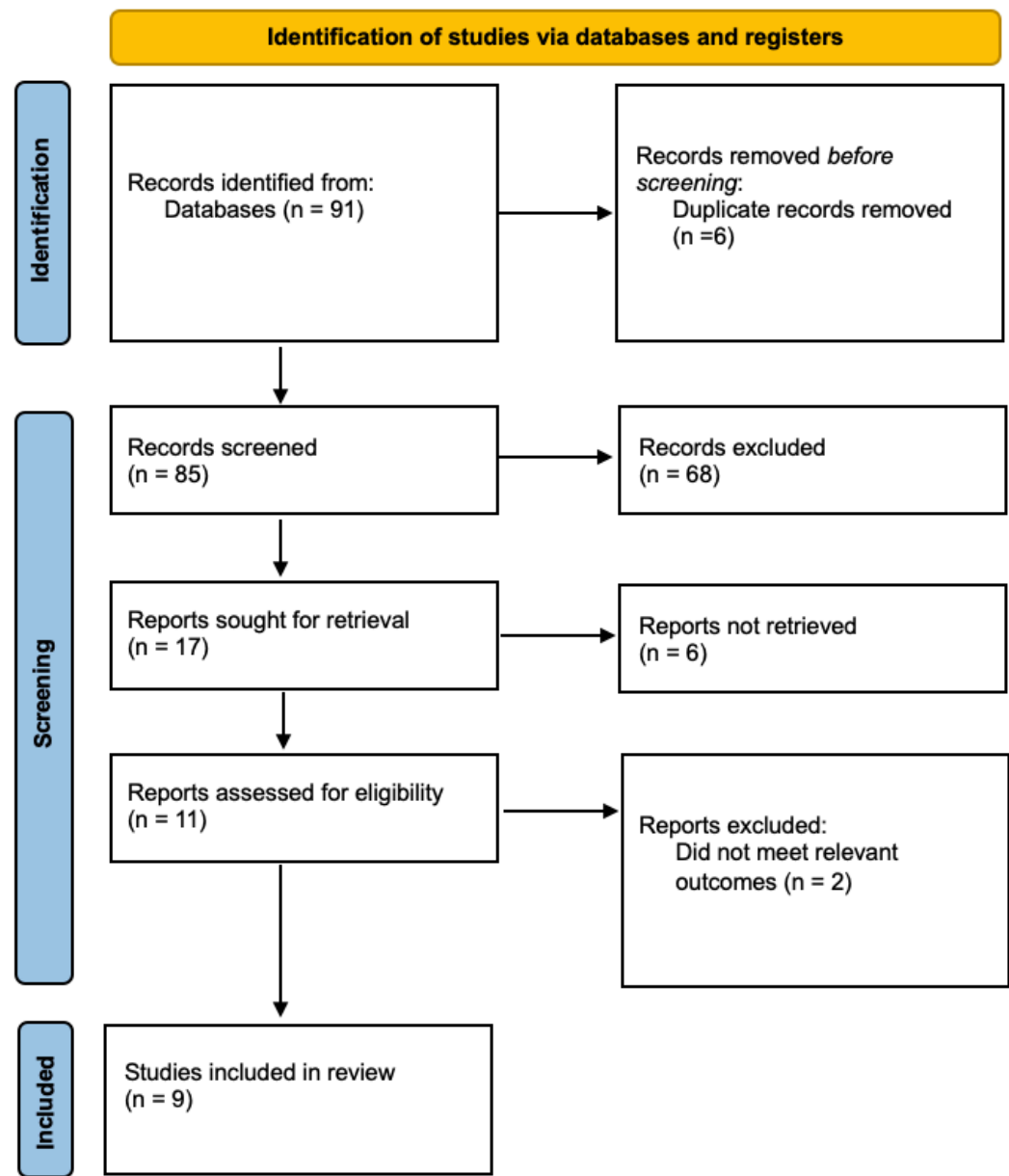


Table 2. *Articles that met exclusion criteria*

Identity Articles	Population of Study	Study Design	Variables/Outcomes
Allar et al. 2022	-patients between 50-75 years who has primary care visit w/in last two years	-retrospective review -multivariate logistic regression	-undergoing CRC screening -self reported race from census -tobacco use -insurance type -tobacco use -obesity -age -high risk of CRC -Marital status -severe mental health disorders -substance use disorder
Doherty et al. 2022	Those w/ cancer (or had cancer) who work -25+	-survey -descriptive stats -univariate logistic regression analyses -multivariate logistic regression analyses	-demographics -cancer type -cancer treatment type -time since diagnosis -health insurance type -employment -cost-related nonadherence
Gyldenvang et al. 2022	-patients -physicians -clinical nurse specialists	-qualitative and quantitative -questionnaire for patients -survey for physicians -two focus group interviews for clinical nurse specialists	-descriptive statistics for patient survey -descriptive stats for physician survey Themes from focus groups -advanced role in nurse-led consultations embraces patient-centered approach -autonomy enhances professionalism -thorough planning of nurse-led consultations provides a strong foundation

Identity Articles	Population of Study	Study Design	Variables/Outcomes
Helweg-Larsen and Tjitra 2024	Smokers n=277	-experimental design -2x2 factorial design	-concealment -ostracism -manipulation check -threat appraisal (questionnaire) -self reported stress (questionnaire) -cognitive depletion (stroop color word inference test) -rejection sensitivity (smoking rejection sensitivity scale) -percieved risk of lung cancer (question) -positive cognitions about smoking (5 items) -self-exempting beliefs (8 items from self-exempting beliefs scale) -interest in smoking cessation website (3 items and seconds spent on site) -smoking cessations intentions (3 items) -self-efficacy (one item) -internalized stigma (six items from internalized stigma of smoking inventory)
Hoyt et al. 2023 (Do not have access thru duke online)	LBG and heterosexual cancer survivors	All of Us database	-physical health-related quality of life -mental health-related quality of life -social health-related quality of life

Identity Articles	Population of Study	Study Design	Variables/Outcomes
Malcarne et al. 2022	-latinos w chronic disease and associated risk -18-75 yrs	-used data from previous study -descriptive analyses	-scale of ethnic experience -simpatia scale -marianismo beliefs scale -sabogal familism scale -brief perceived ethnic discrimination questionnaire-community version -short acculturation scale for Hispanics -sociodemographic variables
Murray et al. 2022	Breast cancer patients -18 years +	-observational -combination of three questionnaires	-participant demographics -questionnaires (adapted versions of exercise identity scale, exercise avoidance scale, and gear of physical activity/exercise scale – breast cancer)
Park et al. 2021	-those w/ alzheimer's and related dementias -45-75 yrs	-questionnaire -self reported physical activity assessment -reviewing old data	-physical assessment questions (many variables) -demographic info -education -medical history -weight and height -smoking -dietary habits (food frequency questionnaire)
Robertson et al. 2022	Older women (65-74 yrs)	-Qualitative-semi-structured interviews -thematic content analysis	-All PLEXs (playful experiences) but control -Value derived from the challenges - Acceptability of the challenges
Santiago-Rodriguez et al. 2022	-sexual and gender minority individuals	-Descriptive analyses -multivariable logistic regression models	-cancer risk behaviors -cancer beliefs -sociodemographic variables -health care access

Identity Articles	Population of Study	Study Design	Variables/Outcomes
Simon et al. 2021	-patients at biobanks	-experimental design -intervention vs control	-understanding (QuIC measure) -confidence in understanding (adapted QuIC measure) -enrollment -demographics
Stephens et al. 2022	Hematology cancer patients -50-70 yrs	-qualitative -interpretive description -interviews	-unique cancer-self -invasion of cancer opposed to self -personification of cancer w/in self
Ussher et al. 2021	-oncology medical practitioners -nurses -allied health professionals -individuals working in leadership roles in cancer care, health and preventative agencies	-Online survey -semi-structures interviews -qualitative and quantitative	-attitudes towards lgbtqi cancer care -knowledge of lgbtqi health need -lgbtqi inclusive practice behaviors Qualitative themes -systemic barriers -consequences of barriers -improving care
Werner-Lin et al. 2022	-families and individuals w/ genetic risk (Li-Fraumeni syndrome in study)	-open ended interviews -modified grounded theory	-new awareness of material body -considering actionability -thresholds of embodiment, disembodiment, and quality of life
Willis et al. 2023	-copd individuals, native hawaiians and filipions	-telephone survey	-e-cig use -cig smoking -financial stress -second hand smoke exposure -bmi -respiratory disease

Identity Articles	Population of Study	Study Design	Variables/Outcomes
Zhang et al. 2022	Representative sample (n=2552) with Asians, Hispanics, and non-Black Hispanics oversampled	Online Survey	-Delaying/forgoing care -experienced racial discrimination -race-related cyberbullying experience -beliefs towards Coronavirus racial bias
Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Anderson et al. 2022	Latina breast cancer survivors	-demographic and short form-36 surveys -semi-structured interviews -thematic analysis -descriptive coding -comparative methods	-quality of life assessment (SF-36) Themes -work adaptation -workplace resilience -family roles and support -maintaining QOL through BCRL self-management
Arefian et al. 2023	Women w/ breast cancer	-questionnaires -correlation -cross-sectional descriptive	-stress (perceived stress scale) -depression (beck depression questionnaire-2) -mindfulness (short form of Freiburg mindfulness questionnaire) -resilience (connor Davidson resilience scale) -spirituality (paloutzian and ellison's questionnaire) -pain (pain intensity measurement and pain interference assessment)
Bhatele et al. 2022	Individuals w/ parkinsons or alzheimers	-use dataset -confused	-confused -math and neuro heavy

Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Boehmer et al. 2021	Colorectal cancer survivors of different sexual orientations	-self report questionnaire -follow-up -logistic regression	-receipt of follow-up surveillance procedures -clinical information (cancer site, stage at diagnosis, date of diagnosis) -self reported health -demographic info (most important is sexual orientation) -social isolation (loneliness scale) -emotional, information, tangible/instrumental support -perceived discrimination experiences -survivors coping styles (brief cope scales) -survivors resilience (resilience scale) -physician and practice level factors -survivorship care plans
Boyer et al. 2022	Patients w/ schizophrenia	-data analysis -differences-in-differences approach -multivariate patient-level logistic regression models	-difference in ppl with schizo cs ppl w/o mental disorders in the pre- vs during covid-19 periods in 90 day hospital non-covid-19 mortality -30-day hospital mortality -patient case mix
Caceres-Matos et al. 2023	Adults with chronic non-cancer pain 18+	-Cross-sectional -questions asked by researcher -data analysis	-impact of chronic non-cancer pain (PAIN_Integral Scale) -demographics

Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Cerezo et al. 2023	Breast cancer patients	-correlation, association, relationship -questionnaire	-life satisfaction (satisfaction w/ life scale) -positive/negative affect (negative and positive affect scale/positive and negative affect scale) -emotional intelligence (short version of trait meta-mood scale) -resilience (connor-davidson resilience scale) -self esteem (self-esteem scale) -optimism (life orientation test-revised) -flourishing (flourishing scale) -depression, anxiety, stress (depressive, anxiety, and stress scale)
Chesak et al. 2023	Family caregivers of individuals w/ head and neck cancer	-intervention	-Feasibility -acceptability (was it worth it questionnaire) -effectiveness of intervention -stress (perceived stress scale) -self compassion (self-compassion scale-short form) -resilience (connor-davidson resilience scale) -anxiety symptoms (patient-reported outcomes measurement information system short form) -mindfulness (mindfulness attention awareness scale)
Deshields et al. 2022	Cancer patients and caregivers	-questionnaire and self report measures	-national comprehensive cancer network distress thermometer -patient-reported outcomes measurement information system anxiety and depression measures -brief resilience scale

Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Dong et al. 2023	Patients w/ bladder cancer	-cross sectional -self report questionnaires and scales	-disease related info -resilience (connor Davidson resilience scale) -uncertainty of disease (mishel uncertainty in illness scale) - trait coping strategies (medical coping modes questionnaire) - perceived social support (social support rating scale) -subjective satisfaction of family functions (family APGAR index) -hope in clinical (herth hope index) -
Dowling et al. 2022	Cancer patients in ireland	Qualitative longitudinal study -semi structured interviews -thematic analysis	-Distress (national comprehensive cancer network distress thermometer) -resilience (connor-davidson resilience scale) Themes -being careful, keeping safe and feeling safe -shrinking supports, feelings isolated and being silenced -not missing out

Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Dunn et al. 2021	Volunteers	Self report survey	-satisfaction w volunteering (satisfaction and personal fulfilment scale) -commitment (organizational commitment questionnaire) -psychological sense of connection (adapted scale) -event and cancer control motives (23 item scale by Won et al. 2010) -social norm (adapted others' expectations scale) -capacity to volunteer -satisfaction w community (1 item from Australian unity wellbeing index) -intention to return (8 item scale)
Gundogmus et al. 2022	Women w/ breast cancer	-relationship, correlation -psychometric properties investigation	-psychological development/growth following a traumatic experience (post-traumatic growth inventory) -psychological resilience (connor davison psychological resilience scale) -psychological resilience (brief resilience scale) - ptsd checklist for DSM-V -anxiety and depression in individuals w/ physical illness (hospital anxiety and depression scale)

Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Hendrix et al. 2023	Firefighters	-intervention study	-depression -anxiety - stress -ptsd -substance use -suicide risk -willingness to attend couple's therapy -quality of life
Hirano et al. 2023	Healthy older adults	-fMRI -experiment -association -dataset	-power to live questionnaire -rosenberg self-esteem scale
Klainin-Yobas et al. 2022	Cancer survivors	-quasi experimental -8 week program -self-reported questionnaires and physiological measures	-psychological well-being (psychological-well being scale) -objective stress (peripheral skin temperature) -subjective stress, depression, and anxiety (depression anxiety and stress scale) -perceived relaxation (perceived relaxation scale) -mindfulness (mindful attention awareness scale) -resilience (connor-davidson resilience scale)
Leung et al. 2022	Chinese family care partners of older adults	-qualitative -interviews -theme analysis	-legislative and cultural norms/ constraints of family, work, and society -CFCP's perseverance to prevent disruptions w/ limited resources -quality of CFCPs' and older Adults' relationships and their trajectory of illness

Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Levedahl et al. 2022	Ppl w/ systemic mastocytosis	-qualitative -semi structured interviews -content analysis	Themes -persistent presence of the disease -struggling against ignorance -an illness of wellness perspective
Liang et al. 2022	Chinese patients w/ an enterostomy	-cross sectional -descriptive -structural equation model analysis -questionnaires	-psychological resilience (10-item resilience scale) -stability of self-concept (self-concept clarity scale) -level of social support in China (social support rating scale)
Luo et al. 2022	Adults w/ colorectal cancer	-descriptive qualitative -interviews	Themes -seeking motivations to move forward -striving for normality -adapting and managing self -drawing on external supports -redefining self
Ma et al. 2023	Family caregivers of patients w cancer	-descriptive phenomenological -unstructured open-ended interviews -theme analysis (colaizzi's seven-step data analysis method)	Themes -feeling scared for the patient -living a life feeling trapped under covid-19 surveillance -feeling neglected and unseen -growing resilience and appreciation
Macia et al. 2022	Cancer patients	-correlational -questionnaires	-emotional control (courtauld scale of emotional control) -resilience (connor-davidson resilience scale) -detect diagnosable psychiatric disorders (general health questionnaire)

Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Mulholland et al. 2023	Breast cancer survivors that went thru chemo -35-73 yrs	-correlation	-fMRI data -spontaneous functional time series -cortical thickness -APOE genotype (saliva) -depression, anxiety, and fatigue (clinical assessment of depression) -cognitive symptoms
Nielsen et al. 2022	Caregivers of adults w/ hematologic malignancies	Qualitative longitudinal study -semistructured interviews -thematic analysis	-committing to an unconditional mission while adjusting to a changeable caregiving role -being an invisible inseparable sufferer -balancing between sacrificing one's own needs and self-care
Santiago et al. 2022 (unsure)	Alzheimer's patients	-gene analysis	-swim analysis -pathway analysis -transcription factor analysis - protein-chemical interaction analysis
Shimizu et al. 2021	Family caregivers of patients with cancer after bereavement	survey	-pre-loss connor-davidson resilience scale -pre- and post-loss Patient Health Questionnaire-9 -post-loss Brief Grief Questionnaire -Posttraumatic Growth Inventory
Smith et al. 2022	Health care professionals	-qualitative online document -framework analysis	Themes -acceptability -fidelity of receipt -contextual fit
Tanriverdi and Turan 2023	Spouses of cancer patients	-descriptive study -online survey	-patient info form -resilience scale for adults -experiences in close relationships scale -hendrick sexual attitude scale

Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Upenieks 2021	Cancer survivors	-dataset use -data analysis	-psychological distress -childhood religiosity -cancer diagnosis -age at diagnosis
Walston et al. 2023	-older adults	-dynamic stimulation tests -deep phenotyping of physical and cognitive function -I'm confused about study design (novel study design)	-baseline contributors -stressor characteristics -static surrogate measures -dynamic stimulation indicator measures -resilience phenotype measures -clinical outcomes
Wen et al. 2023	Surrogates of terminally ill cancer patients	-longitudinal and observational	-decisional regret (decision regret scale) -decisional-regret trajectories (latent-class growth analysis)
Wong et al. 2022	Older adults -3 senior investigators in aging in study	-personal stories -future research	-NA
Self-Concept Articles	Population of Study	Study Design	Variables/Outcomes
Abbas et al., 2022	Cancer patients during chemotherapy	-therapy interventions (cbt)	-depression -anxiety -stress -death anxiety -satisfaction with life -self-esteem

Self-Concept Articles	Population of Study	Study Design	Variables/Outcomes
Bahnsen et al., 2022	-adults w/ sexual inactivity and dysfunctions	-dataset -correlation	-inter-personal sexual inactivity within the last year -sexual dysfunctions within the last year -hypoactive sexual desire disorder within the last four weeks -International index of erectile function -female sexual function index -self-rated health -physical and mental health problems
Boding et al., 2022	Women w/ ovarian cancer	Survey -responses analysed thematically	Themes -Failure and loss of femininity and womanhood -Internalising public perceptions of the self -altered relationships
Castro-Vazquez et al., 2023	Japanese urologists	Interviews	Themes -An onco-self -Onco-bipedagogy -onco-economics
Chui et al., 2022	Head and neck cancer patients	-cross-sectional -questionnaires -phone interviews	-return to work barrier scale -return to work facilitator scale -distress thermometer -numeric rating scale -karnosky performance status scale -demographic and clinical characteristics

Self-Concept Articles	Population of Study	Study Design	Variables/Outcomes
de Almeida et al., 2022 (link does not take you to article)	Cancer patients undergoing exclusive palliative care in regard to death	-descriptive -exploratory -cross-sectional -qualitative	-observations recorded in field diary (social support network, ways of coping, perception and reaction to death process, autonomy) -free word association test
Emanuel et al., 2023	Adults w/ cancer receiving palliative care	Questionnaires correlations	-Death anxiety and distress scale -demographic factors -religious struggle -dignity-related distress -existential quality of life -terminal illness awareness
Enyi and Mousavi, 2023	-hospitalized patients with cancer	-descriptive, correlational, structural equation modeling -questionnaires	-self-perceived burden questionnaire -cognitive fusion questionnaire -uncertainty intolerance scale -cancer behavior scale
Fennel et al., 2023	Adults recruited through CloudResearch	-self reported questionnaire over multiple times -assigned groups seeing different priming info before questionnaires on follow-ups	-sun exposure -sun exposure intentions -resource engagement -self-esteem -momentary self-worth -narrow self-assessment -defensiveness

Self-Concept Articles	Population of Study	Study Design	Variables/Outcomes
Joosten et al., 2023	-adult siblings of long term survivors of childhood cancer	-questionnaires	-health related quality of life (TNO-AZL questionnaire of Adults' HRQoL) -Anxiety and depression (hospital anxiety and depression scale) -Post-traumatic stress symptoms (self-rating scale of post-traumatic stress disorder) -self-esteem(rosenberg self-esteem) -benefit finding and disease related burden (benefit and burden scale for children)
Kiley-Morgan et al., 2021	-adult patients w/ cancer	-intervention	-anxiety and depression (hospital anxiety and depression scale) -self-esteem (Rosenberg self-esteem scale) -open ended qualitative questions
Lohaus, 2023 (not available online)	Multiple sclerosis and stroke patients	-quantitative	-socio-cognitive test battery -questionnaires on fatigue and psychopathology
Perrier et al., 2021	Breast cancer patients	-fMRI -questionnaires	-anxiety (state-trait anxiety inventory) -depression (beck depression inventory short form) -self-referential (questionnaire of self-representations) -stability of trait self-knowledge (certainty of self-concept score) -global-self esteem -fMRI data on resting state in dmPFC, PCC, and dorsal ACC

Self-Concept Articles	Population of Study	Study Design	Variables/Outcomes
Shen et al., 2022	Breast cancer survivors	Multicentre cross-sectional study -self reported questionnaires	-socio-demographic characteristics -disease and treatment-related characteristics -lymphedema self-management behaviors -lymphedema knowledge -illness perception -self-efficacy -self-regulation -social support
Sun et al., 2022 (not available through duke online)	UK biobank participants and FinnGen participants	-quantitative	-genetic associations -biomarkers
Suppiah et al., 2023	-older adults (Singaporean)	-national survey dataset	-health literacy (BRIEF instrument) -socio-demographic variables -health related variables
Weis, 2022 (not available online)	Men w/ cancer and men that were relatives or close friends of people w/ cancer	-questionnaires -interviews	-distress thermometer -patient health questionnaire -generalized anxiety disorder scale -short form Health Survey -modified questionnaire for dance therapy -interviews: expectations of project, experience of rehearsals and dance group, coping with cancer experience, distress and limits of project, utilization of psycho-oncological support, expectations for performance, return to normality of life, experienced resources

Self-Concept Articles	Population of Study	Study Design	Variables/Outcomes
Xiao et al., 2022	-adults from national health and nutritional examination survey	-dataset	-physical activity measurement and fPCA-based rest-activity profiles -participant characteristics
Yamazaki et al., 2023	Japanese patients with advanced cancer	Questionnaires Correlations	-Quality of Life at the End of Life-Cancer Scale -Generalized Anxiety Disorder-7 -Rosenberg Self-Esteem Scale -Multidimensional Scale of Perceived Social support -Functional Assessment of Cancer Therapy-General Scale -Functional Assessment of Chronic Illness Therapy-Spiritual
Zhai et al., 2021	Chinese women w/ breast cancer	-semi structured interviews	Themes -renewing self-perception -encountering changes in relationships -altering philosophical values and beliefs

Table 3. *Articles that met inclusion criteria*

Identity Articles	Population of Study	Study Design	Variables/Outcomes
Bybee et al. 2023 (Do not have access thru duke online)	-sexual and gender minority couples where one partner had cancer diagnosis at some point in life -heterosexual couples where one partner had cancer diagnosis at some point in life	-questionnaires -interviews	-demographics -post traumatic growth (PTG) domains -individual ptg -dyadic ptg -similarities and differences of ptg between couples -comparison between sgm and non sgm couples -
Ciaralli et al. 2021 *age range from 58-95	-older adult cancer survivors -60 years +	-data from older study -statistical analyses -differences between means -bivariate correlation analysis -three ordinary least-squares regression analyses -regression analyses	-perceived disability (1 item) -demographic and personal characteristics -# of comorbidities -level of reported functional difficulties (nagi 1976 index) - # of current non-cancer symptoms -# of treatment types -cancer stage -current cancer symptoms

Identity Articles	Population of Study	Study Design	Variables/Outcomes
Cubis et al. 2023	-ppl with brain tumors - 22-69 yrs (35 months post diagnosis in study)	-qualitative -social identity mapping -in depth semi-structured interviews	Theme 1: engaging and connecting -barriers -functional impariments -fear of negative evaluation -discomfort of others -facilitators -strategies Theme 2: then vs now -stability -maintenance and expansion -loss and rebuilding -loss and shrinking
Dahlberg et al. 2021	-individuals w brain tumors -34-66 yrs -study included informal caregivers (25-88 yrs) and bereaved caregivers (27-54 yrs)	-qualitative -interviews, audio recorded and transcribed	-percieved usefulness of the CareMaps tools -potential areas of use -capturing the complexity of social relations -timing for introduction of the CareMaps tool -self-care supportive relations -identity-preserving relations
Harmon and Kyle 2019	-cancer or remission patients average age 67 yrs -two males and ten females	-semi-structured interviews based upon prior informal discussion	-historical relationship to nature and leisure -diagnosis of cancer and accompanying treatment program -participation in hiking program -outlook for future

Identity Articles	Population of Study	Study Design	Variables/Outcomes
Li et al. 2022 *breaks down by age (60-82 cohort is target for us)	-breast cancer survivors	-descriptive correlational design	-sociodemographic and clinical characteristics -illness perceptions (revised illness perception questionnaire) -adherence to BCRL risk management behaviors (lymphedema risk management behavior questionnaire)
Martin et al. 2023 *note that two participants were in 40s and three were between 50-65	-those who had completed for bowl cancer	-interpretive descriptive qualitative methodology -inductive analytic approach	Themes -I had to heal myself -Life's too short -The person I've become
van der Kamp et al. 2022 *one participant is 47, six are 50-65	-men w/ prostate cancer	-narrative analysis	Themes -reconstructing identity -shifting relationship w/ prostate cancer -relationship w/ technology -uncertainty, incoherence and haunting
Wang et al. 2024	Postmenopausal women (50-79 yrs)	-Quantitative -Associations	-Invasive breast cancer incidence in Feb 2022 follow-up -Breast cancer molecular subtypes -Allostatic load score (based upon 8 submarkers)

Identity Articles	Population of Study	Study Design	Variables/Outcomes
Yeung et al. 2022 Mean age is 68.75 SD=10.66	-rural patients w/ advanced cancer -mean age 68.75 yrs	-qualitative descriptive design -semi-structured interviews w open ended questions	-demographic info -clinical and disease info Themes -fluidity in patients' emotions -religion/spirituality -social support -positive attitude -cognitive strategies -self-care -medication
Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Chen et al. 2021	-Cancer patients in Taiwan -M=67.18 yrs; SD=10.82; Range = 42 yrs – 87 yrs	-Advanced Care Plan	-coping scale ways of coping checklist-revised -social support scale -resilience scale
Chien et al. 2022 *age range from 46-84, but median=69 and mean=68.82, SD=6.74	Prostate cancer survivors	-cross-sectional -self reported questionnaires	-disease characteristics -physical symptoms (mandarin version of expanded prostate cancer index composite) -cancer specific self-efficacy (mandarin version of cancer survivors' self-efficacy scale) -psychological resilience (mandarin version of connor-davidson resilience scale) -demoralization (mandarin version of the demoralization scale)
Irwin et al. 2022 *ages from 55-85	Long term breast cancer survivors -55-85 yrs	-associations, correlations	-time to incident or recurrent major depressive disorder -level of interleukin-8 at enrollment

Resilience Articles	Population of Study	Study Design	Variables/Outcomes
Michael et al. 2022 *age range 44-99, but mean=71.8, SD=11.4	Cancer patients	-video intervention vs standard -quantitative and qualitative	Theme -cancer patients ability to posture vulnerability w/ resilience Subthemes -acknowledging frailty w/ diverse adaptations to dependency -benevolence and reciprocation of family/friends caring -death anxiety, ambivalence and experiential acceptance of dying -honouring death through secular and sacred rituals
Self-Concept Articles	Population of Study	Study Design	Variables/Outcomes
Bluck et al., 2022 *age range 55-75	Older adults with late stage cancer	-self report questionnaires -therapy intervention -narrative analysis	-demographics and health status (Edmonton symptom assessment, palliative performance scale) -dignity-related distress (patient dignity inventory) -spiritual distress (religious/spiritual struggles scale) -quality of life at end of life -narrative analysis of therapy sessions
De La Torre et al., 2022 *associations based upon age	Cancer survivors	-dataset (survey) -associations	-physical activity (number of minutes of moderate to physical vigorous activity MVPA) -perceived health competence -perceived social relatedness for health -wearable activity trackers -self-related health

Self-Concept Articles	Population of Study	Study Design	Variables/Outcomes
Törnävä et al., 2021 *age range 50-91	Men w penile cancer surgery	Interview or response letter -thematic analysis	Themes -cancer-modified me -everyday life defined by physical symptoms -sexual life defined by cancer -reshaped content of life

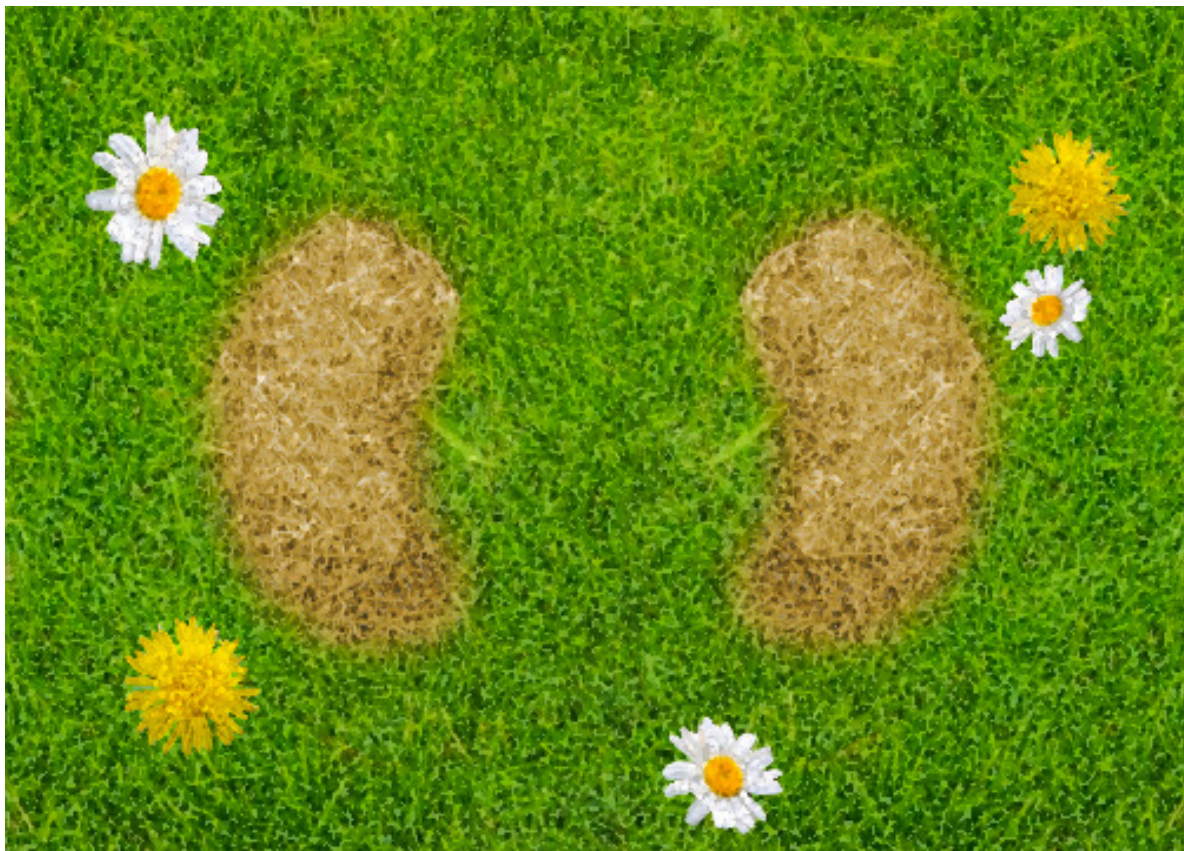
**Investigating a link between Glyphosate and Chronic Kidney Disease
of Unknown Origin (CKDu) through Glyphosate's interactions with
Renal Proteins in Molecular Docking.**

Vignesh Pirapaharan



Article Synopsis

This study aims to investigate the underlying mechanism of Chronic Kidney Disease of Unknown Origin. Given that glyphosate concentration presents a high correlation with the diagnosis of the disease, glyphosate's binding affinity with the renal proteins were studied using molecular docking. Molecular docking analyses the intermolecular interactions between the renal protein candidates and the ligand (glyphosate) and is able to shortlist the protein candidates for laboratory testing of the pathways associated with the protein.



Graphic by Gaby Dunn

Investigating a link between Glyphosate and Chronic Kidney Disease of Unknown Origin (CKDu) through Glyphosate's interactions with Renal Proteins in Molecular Docking.

Vignesh Pirapaharan

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ABSTRACT

Chronic Kidney Disease of unknown origin (CKDu) is a subtype of chronic kidney disease that is mainly observed in agricultural communities in Sri Lanka, India, and parts of Central America. The cases of CKDu in agricultural settings are correlated with the use of pesticides. Among these, glyphosate, an herbicide, has been shown to correlate with CKDu cases in Sri Lanka. Various mechanisms have been proposed for glyphosate's association with CKDu including the inhibition of CYP450 enzymes and the disruption of the renin-angiotensin-aldosterone system. Given that glyphosate's mode of action involves the binding to and inhibition of the protein EPSP synthase, this study employs molecular docking to identify renal protein candidates with favorable binding interactions with glyphosate at an atomic level. The results revealed that renal proteins such as interleukin-6, uromodulin, and prorenin exhibited energetically favorable binding with glyphosate, potentially influencing the prognosis of CKDu. In the future, it is necessary to experimentally validate these findings through in-vitro studies to confirm glyphosate's binding with these proteins of interest. The experimental validation could perhaps lend insights into the etiology of the CKDu.

INTRODUCTION

Chronic Kidney Disease (CKD) is characterized by the presence of kidney damage or an estimated glomerular filtration rate (eGFR) of less than 60ml/min per 1.73m². The chronicity of CKD is established when it lasts for more than 3 months (Vaidya & Aedulla, 2022). While many possible factors may contribute to the development of CKD, the most prevalent risk factors are Diabetes Mellitus Type 2 and Hypertension. Common symptoms of CKD include nausea, vomiting, loss of appetite, peripheral edema, muscle twitching, and resistant hypertension. As of 2022, the global prevalence of CKD is estimated at 9.1% to 13.4% contributing to approximately 1% of disability-adjusted life years (DALYs) — the sum of years lost due to living disability and premature mortality— and 1%-3% of life years lost in 2012 (Kovesdy, 2022).

Chronic Kidney Disease of unknown origin (CKDu) is a subtype of chronic kidney disease predominantly

observed in agricultural communities in Sri Lanka, India, and certain regions of Central America (Anupama et al., 2020). Patients diagnosed with CKDu typically exhibit non-diabetic, low or minimal proteinuria (elevated protein amounts in urine), and normal or mild hypertension, thereby excluding these factors as primary contributors to the chronic kidney disease (Anupama et al., 2020). In El Salvador and Sri Lanka where CKDu is prevalent, pesticides such as MCPA (2-methyl-4-chlorophenoxyacetic acid), pretilachlor, propanil, glyphosate, paraquat, and hedonal are used. It may be beneficial to isolate a specific pesticide for future investigations (Jayasiri et al., 2021; Mejia et al., 2014). Additionally, it was estimated from a 2023 study that subjects who were in the highest tertile of exposure duration to indoor pesticides had 1.64 times higher odds of being diagnosed with CKDu (Alvand et al., 2023).

Chemical analysis of well water in Sri Lanka has

indicated that glyphosate is the pesticide most strongly correlated with CKDu prevalence in this region (Ulrich et al., 2023). Glyphosate is an herbicide with the empirical formula $C_3H_8NO_5P$, containing phosphate, carboxylic acid, and amino group (Figure 1). Glyphosate is used prior to planting and post-harvest to clear fields (Benbrook, 2016). Urinary analysis in Sri Lanka has revealed that glyphosate residues were in higher concentrations among farmers compared to non-farmers (Abdul et al., 2021). Furthermore, this study found a correlation between urinary glyphosate levels and CKDu cases, while additional research indicated that glyphosate may play a role in renal damage observed in CKDu patients (Gunatilake et al., 2019; Abdul et al., 2021).

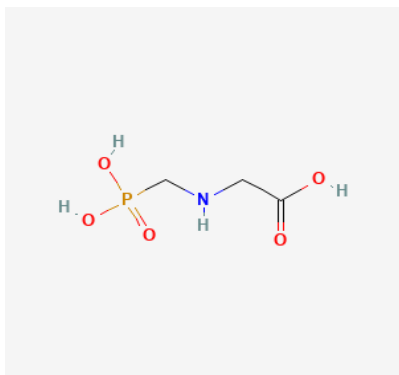


Figure 1. The structure of glyphosate. The phosphate group is located on the left side of the molecule, the amine group is positioned centrally, and the carboxylate group is situated on the right side (PubChem).

Previous studies have proposed some mechanisms of renal toxicity that are caused by glyphosate. Glyphosate inhibits cytochrome P450 (CYP) enzymes, which are crucial for detoxifying xenobiotics in the liver and kidney (Samsel and Seneff, 2013; Knights et al., 2013). Moreover, glyphosate has also been shown to facilitate the transport of arsenic beyond the gut barrier to the kidneys. The acidic environment of the urine may induce stress in renal tubules, potentially contributing to kidney disease (Jayasumana et al., 2014; Jayasumana et al., 2013; Gunatilake et al., 2019).

Additionally, glyphosate can cause heart damage through mechanisms involving cellular senescence

and the reduction of proliferating cells (Lu et al., 2023). This cardiotoxicity is also associated with disruption of the renin-angiotensin system — responsible for water and sodium homeostasis — by organophosphates such as glyphosate (Rajak et al., 2022). This disruption is noteworthy as approximately 80% of CKD patients are hypertensive; with this hypertension often attributed to the hyperactivity of the renin-angiotensin system (Santos et al., 2012).

Despite all the proposed mechanisms of toxicity, the specific renal proteins most affected by glyphosate remain unidentified, representing a critical knowledge gap in the field. Identifying these proteins will facilitate the investigation into their functions and provide insight into the process disrupted by glyphosate exposure. Also, the identification of renal proteins which are likely to be affected will inform future studies, narrowing down the list of proteins for *in-vitro* analyses. Furthermore, by narrowing down the list of proteins for *in-vitro* analyses, the time and money spent on researching unlikely pathways is reduced and research groups could do more focused research on pathways concerning the renal proteins identified. Therefore, it is important to examine which renal proteins undergo favorable binding interactions with glyphosate (Yue et al., 2008; Schonbrunn et al., 2001).

Previous research primarily focused on proteins such as acetylcholinesterase (ACHE), adenosine monophosphate-activated protein kinase (AMPK), and cytochrome P450 (CYP450), whose effects extend beyond the kidney (Rajapaksha et al., 2021). This study will instead concentrate on renal proteins that have not been previously examined. These proteins were identified through literature review and RCSB protein bank, followed by molecular docking to assess glyphosate binding to these targets. The docking process involves two steps: assessing the conformation of ligand and orientation within the binding sites, followed by evaluating the binding affinity with the ligand upon interaction (Meng et al., 2011). The binding affinity is determined through the analysis of various intermolecular interactions, including Van der Waals, hydrogen bonding, hydrophobic interactions,

and electrostatics (Mohanty & Mohanty, 2023). The outcomes of this study will help predict which proteins are most affected by glyphosate, providing a shortlist for *in vitro* analysis.

METHODS

Obtaining Proteins of Interest

Suitable protein candidates were identified through a literature review using PubMed and RCSB protein data bank. Search terms included “kidney disease,” “kidney,” and “chronic kidney disease” to compile a list of relevant proteins. A filter was applied to ensure that only proteins applicable to *Homo Sapiens* were considered. The search results were examined to confirm that the identified proteins were associated with kidney. This approach yielded a total of 69 protein candidates.

Molecular Docking

Autodock Tools 1.5.7 Autodock 4.2.6 were used to conduct and analyze the molecular docking process. The .pdb files of the candidate proteins were obtained from RCSB Protein Data Bank and the ligand's, or glyphosate's, structure was obtained from PubChem using the SMILES notation. Then the ligand's structure was converted to .pdb format using Babel. After importing the protein .pdb file into AutoDock, water molecules were removed, hydrogen atoms were added, and Kollman charges, or partial atomic charges, were assigned. To clarify, the RCSB Protein Data Bank is a repository of protein structures, SMILES notation is a method usually used to represent an organic molecule, and Babel is an online program used to convert file formats of molecules. The edited protein was saved as a .pdbqt file. Next, the ligand was imported into AutoDock Tools, and its torsions were adjusted using the torsion tree before saving it as a .pdbqt file.

Then, the GRID box was used to load the edited protein, and the “set map type” button was pressed to integrate the ligand. To clarify, the GRID box is a 3-dimensional coordinate system used to focus on the portion of interest of the protein. The GRID box was adjusted to cover both the ligand and the protein, promoting blind docking to identify

potential binding sites. The GRID output was saved as a .gpf file. AutoGrid was then executed using the saved .gpf file. The rigid file name was set to the protein's pdbqt file, and the ligand was chosen from the popup menu. The docking process utilized the Lamarckian method, which is a molecular docking method that is used to estimate the best binding zones between the ligand and the protein, with the output saved as a .dpf file. Molecular docking was then initiated by selecting the .dpf file, and the resulting .dlg file was generated for analysis.

These steps produced binding energy values for each protein-ligand interaction, which were then compiled for subsequent statistical analysis. The mean binding energies for the 10 conformations were then calculated to assess the relative binding affinities of the proteins with the glyphosate ligand. Finally, plots and figures were generated using R 3.3.0+.

RESULTS

Among the 69 proteins examined, 32 exhibited binding energies less than 0 kcal/mol, indicating relatively favorable interactions. Glyphosate's binding with EPSP synthase in *Arabidopsis Thaliana* was selected as a reference, as glyphosate is a known inhibitor of this enzyme. While glyphosate concentrations are typically higher in plants than in serum, the binding interaction with EPSP synthase was assessed to evaluate the feasibility of glyphosate in *Arabidopsis Thaliana* (van Bruggen et al., 2021 & Gillezeau et al., 2019).

To identify the most susceptible proteins to glyphosate, a cutoff of -0.562kcal/mol was selected based on the binding energy between glyphosate and EPSP synthase (the enzyme inhibited by glyphosate when it acts as a herbicide). The results are shown in Figure 2.

Table 1: The proteins of interest with their RCSB code and their description/functionalities with their binding energies. The blue rows indicate the proteins that have the most susceptible binding to glyphosate based on their binding energies being less than the reference (the green row)

Protein Identity (RCSB Code for the Protein Structure)	Function/Description	Average Binding Energy/ kcalmol ⁻¹ (a negative value indicates higher binding affinity)
1ALU	Structure of Interleukin-6 which is a key player in inflammation in chronic kidney disease	-2.407
4WRN	Crystal structure of the uromodulin/Tamm-Horfsall protein.	-1.316
2H8R	Transcription factor (bound to DNA) that ensures the development and proper function of kidney.	-1.492
5M4V	Structure of Mambaquarentin; Mambaquarentin is involved against Polycystic Kidney Disease.	-0.956
6A70	Combination of the human PKD1 and PKD2 complex for Polycystic Kidney Disease.	2.065
5KL2	A Wilms Tumor Protein Transcription Factor in complex with DNA.	2.080
5KL3	Another Wilms Tumor Protein Transcription Factor in complex with DNA.	2.080
5KL5	Another Wilms Tumor Protein Transcription Factor in complex with formylated DNA.	-0.930
5KL6	Another Wilms Tumor Protein Transcription Factor in complex with DNA.	2.079
5KL7	Another Wilms Tumor Protein Transcription Factor in complex with carboxylated DNA.	-1.464
5T4D	Structure of Polycystic Kidney Disease Protein 2.	2.013
5K47	Structure of Polycystin-2 (Involved in Polycystic Kidney Disease).	2.005
5KL4	Another Wilms Tumor Protein Transcription Factor in complex with formylated DNA.	2.061
6B0O	Substructure of mutated version of the Wilms Tumor Transcription factor in complex with an oligonucleotide.	2.070
6B0P	Substructure of another mutated version of the Wilms Tumor Transcription Factor in complex with an oligonucleotide.	-3.472
6B0Q	Substructure of another mutated version of the Wilms Tumor Transcription Factor in complex with an oligonucleotide.	2.114
6B0R	Substructure of another mutated version of the Wilms Tumor Transcription Factor in complex with an oligonucleotide.	-3.132

Protein Identity (RCSB Code for the Protein Structure)	Function/Description	Average Binding Energy/ kcalmol ⁻¹ (a negative value indicates higher binding affinity)
6BLW	Substructure of another mutated version of the Wilms Tumor Transcription Factor in complex with an oligonucleotide.	-1.841
8D43	Structure of human kidney Glucosidase II.	2.110
7F09	Structure of a transcription factor; when this transcription factor has undergone dysregulation, it becomes associated with renal cell carcinoma.	-1.721
6ZS5	A cryo EM structure of human uromodulin filament core.	2.090
6ZYA	Extended structure of human uromodulin filament core at 3.5A resolution.	2.090
3HRO	C-terminal coil of the transient receptor potential channel of polycystic kidney disease 2.	1.050
4AMT	Crystal structure of human prorenin.	-1.876
3HRN	Another C-terminal coil of the transient receptor potential channel of polycystic kidney disease 2.	-0.713
2REN	Structure of recombinant human renin.	-0.312
3OWN	Structure of potent macrocyclic renin inhibitors; binding interactions were observed to see if glyphosate had any effects with a hypothetical similar structure bodily produced renin inhibitor.	-1.135
3Q5H	A structure of a synthetic renin inhibitor; binding interactions were observed to see if glyphosate had any effects with a hypothetical similar structure bodily produced renin inhibitor.	-0.141
4CH9	Crystal structure of human Kelch domain in complex with a WNK4 peptide.	-1.098
8IVR	Synthetic Structure of a therapeutic against CKDu; binding interactions were observed to see if glyphosate had any effects with a hypothetical bodily synthesized therapeutic against CKDu.	1.493
8IXS	Synthetic Structure of a therapeutic against CKDu; binding interactions were observed to see if glyphosate had any effects with a hypothetical bodily synthesized therapeutic against CKDu.	1.299
1RNE	The crystal structure of recombinant glycosylated human renin.	0.288
2G1N	Structure of a renin-inhibitor.	-0.201
2G1O	Another structure of a renin-inhibitor.	1.762

Protein Identity (RCSB Code for the Protein Structure)	Function/Description	Average Binding Energy/ kcalmol ⁻¹ (a negative value indicates higher binding affinity)
2G1R	Another structure of a renin-inhibitor.	0.464
2G1S	Another structure of a renin-inhibitor.	-0.224
2G1Y	Another structure of a renin-inhibitor.	1.025
2G22	Another structure of a renin-inhibitor.	-0.260
3G6Z	A structure of a synthetic renin inhibitor; binding interactions were observed to see if glyphosate had any effects with a hypothetical similar structure bodily produced renin inhibitor.	0.0954
3G70	A structure of a synthetic renin inhibitor; binding interactions were observed to see if glyphosate had any effects with a hypothetical similar structure bodily produced renin inhibitor.	-0.387
3OOT	Structure of renin-indole-piperazin inhibitor complexes.	0.423
3OQF	Another structure of renin-indole-piperazin inhibitor complexes.	0.051
3OQK	Another structure of renin-indole-piperazin inhibitor complexes.	0.802
3Q4B	Synthetic structure of an alkyl amine renin inhibitor; binding interactions were observed to see if glyphosate had any effects with a hypothetical similar structure bodily produced renin inhibitor.	-0.570
8IVG	Synthetic Structure of a therapeutic against CKDu; binding interactions were observed to see if glyphosate had any effects with a hypothetical bodily synthesized therapeutic against CKDu.	1.870
5KOQ	Structure of Tak-272, an orally active renin inhibitor.	0.272
5TMG	Structure of Orally Active Renin Inhibitor.	-0.267
2PRT	Structure of Wilms Tumor Protein transcription factor with DNA.	-0.569
3VOY	Structure of human glutaminase in apo form.	0.509
4NEF	X-ray structure of human Aquaporin 2.	-0.637
7PFP	Cryo EM structure of native human uromodulin (UMOD)/Tamm Horsfall protein filament.	1.295
3VCM	Crystal structure of human prorenin.	-0.470
8D45	Structure of human Kidney Betaine- Homocysteine Methyltransferase.	2.100

Protein Identity (RCSB Code for the Protein Structure)	Function/Description	Average Binding Energy/ kcalmol ⁻¹ (a negative value indicates higher binding affinity)
4CSK	Structure of Human Aquaporin.	2.057
6DU8	Structure of Human Polycystin 2-I1.	2.09
8D44	Structure of human Kidney Fructose- bisphosphate aldolase B.	2.07
3V42	Structure of renal Tumor Suppressor Protein, Folliculin.	-0.63
8BLO	Structure of human urea transporter.	-0.254
4CHB	Ion transporter complex in kidney involving WNK1 and WNK4.	1.073
2JP8	The compilation of Angiotensin 1-7.	0.3512
2WXW	The crystal structure of human angiotensinogen.	-0.204
8ET6	The structure of the organic cation transporter 1 in the kidney in the apo state.	0.665
4OJ2	The structure of aquaporin.	-0.463
4TNT	The structure of the human mineralocorticoid receptor in complex with DNA. The mineralocorticoid receptor is involved in electrolyte balance.	0.151
1J04	Structure of the enzyme involved in hereditary kidney stone disease.	-1.077
6BZR	ABCC6 in ADP-Bound state; ABCC6 is a novel inhibitor of Vascular Calcification. The levels of 6BZR are reduced in Chronic Kidney Disease.	-0.311
1O8A	Native crystal structure of human angiotensin converting enzyme.	-0.673
2C6F	N-domain structure of full-length angiotensin 1-converting enzyme.	0.147
7Q49	Refinement structure of N-domain structure of full-length angiotensin 1-converting enzyme.	2.110
7PXY	The crystal structure of EPSP synthase in <i>Arabidopsis Thaliana</i> for reference as glyphosate inhibits the EPSP synthase in plants. This value was obtained by the similar methods of docking as other protein candidates.	-0.562

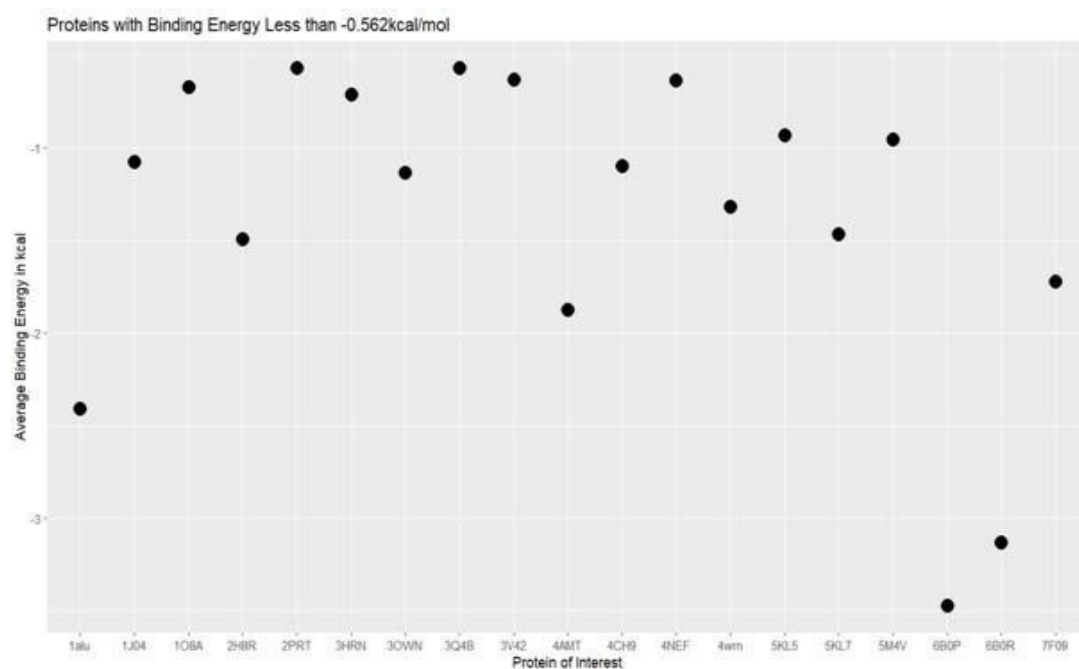


Figure 2: The average binding energy plotted against the proteins of interest to show the proteins with binding energy less than -0.562 kcal/mol. The protein identities are highlighted in Table 1.

DISCUSSION

In this study, 69 candidate protein structures were selected from the protein database and subjected to molecular docking with glyphosate as the ligand. Analysis of the binding energies revealed that 32 proteins (46.3%) exhibited binding energy less than 0 kcal/mol, while 19 (27.5%) proteins had a binding energy below -0.562 kcal/mol. This latter value was chosen based on the binding energy observed in the molecular docking of glyphosate with EPSP synthase in *Arabidopsis Thaliana*. Interleukin-6, Uromodulin, and Prorenin are three candidate proteins that exhibited the low binding energies.

Interleukin-6

Interleukin-6 (1alu) has an average binding energy of -2.407 kcal/mol with glyphosate, indicating a thermodynamically favorable interaction. Interleukin-6 is a cytokine with diverse biological functions, primarily regulating immune and inflammatory response, hematopoiesis, metabolism, and organ development (Su et al., 2017). Additionally, Interleukin-6 speeds up the development of CKD by worsening the inflammation and contributing to complications such as vascular disease (Su et

al., 2017). In CKD, dysregulation of the immune system allows cytokines such as Interleukin-6 to trigger autoimmunity, chronic inflammation, and tissue damage, further aggravating the inflammation process associated with CKD (Jones et al., 2015). Glyphosate's binding to Interleukin-6 may lead to either activation or inhibition of this protein; however, it is worth noting that activated Interleukin-6 could potentially enhance the inflammatory response and exacerbate kidney injury.

Uromodulin

Uromodulin (4wrn) exhibits an average binding energy of -1.316 kcal/mol with glyphosate, indicating a thermodynamically favorable interaction. Lower levels of uromodulin are associated with decreased erythropoietin production and problems in maintaining an acid-base equilibrium (Fedak et al., 2016). A prospective cohort study found that elevated uromodulin levels correlated with a 40% reduction in the risk of developing CKD (Wen et al., 2023). Moreover, lower serum uromodulin levels have been independently linked to end-stage kidney disease (Li et al., 2018). These findings underscore the role of uromodulin in kidney function. Given

that uromodulin circulates in the bloodstream, the probability of glyphosate interacting with this protein is relatively high (Thielemans et al., 2023). Glyphosate's binding to uromodulin may lead to either activation or inhibition of this protein; however, inhibition could contribute to the progression of CKD, as lower uromodulin levels are associated with impaired kidney function.

Prorenin

Prorenin (4amt) has an average binding energy of -1.876 kcal/mol with glyphosate, indicating a thermodynamically favorable interaction. Prorenin is a precursor to renin, a hormone involved in the renin-angiotensin system, which regulates water and sodium homeostasis (Rajak et al., 2022; Danser, 2006). This renin-angiotensin system is closely linked to hypertension, particularly in CKD patients, with approximately 80% of CKD patients exhibiting hypertensive symptoms attributed to hyperactivity of the renin-angiotensin system (Santos et al., 2012).

Glyphosate's binding to prorenin may result in either activation or inhibition of this protein. Given that elevated prorenin levels are linked to hypertensive symptoms, the activation of prorenin by glyphosate might be associated with the progression of CKD.

Glyphosate and its possible relation to other diseases

Molecular docking of glyphosate with various proteins reveals that it not only interacts with proteins associated with CKD, but also with proteins associated with other diseases.

One notable example is the high binding affinity of glyphosate with the mutated Wilms Tumor Protein Transcription Factors such as 6b0p and 6b0r. Non-mutant Wilms Tumor Protein Transcription Factors are critical for the proper development of the urogenital system (Englert, 1998), while the mutant versions are associated with Wilms Tumor, Denys-Drash Syndrome, nephropathy, and abnormalities in genital development (Rauscher, 1993). The binding energy between glyphosate and 6b0p is -3.472 kcal/mol and -3.132 kcal/mol for 6b0r, suggesting that glyphosate might activate these mutant versions of the transcription factors, potentially exacerbating

associated symptoms. Additionally, glyphosate shows a binding affinity for non-mutant Wilms Tumor Protein Transcription Factors such as 5kl5 (-0.930 kcal/mol) and 5kl7 (-1.464 kcal/mol), which could be inhibited, allowing the effects of the mutant variants to prevail.

Though it may seem that a mutant version of Wilms Tumor Protein Transcription Factor would have its effects limited to Wilms Tumor, it is not always the case. A nationwide cohort study in Denmark involving 20-plus-year survivors of Wilms Tumor revealed that out of the 99 Wilms Tumor patients, 19 (19%) of them were classified with CKD with moderate, high, or very high risk (Hogsholt et al., 2023).

Limitations

While molecular docking allows for the assessment of many proteins efficiently compared to individual protein reaction assays, it has notable limitations. A major problem is the relative inaccuracy in prediction, which may arise from challenges in accurately modeling intermolecular interaction within the protein molecule, or within the ligand molecule, or within the protein-ligand bound complex. Thus, it is critical to supplement the results of the docking with in-vitro protein assays (Sethi et al., 2020).

Additionally, the limited number of ligand-receptor conformations explored by docking software due to technological constraints can hinder the calibration of *in-silico* results with experimental results. The ligand could bind at a different active site in the protein which is unexplored by the docking software (Pinzi & Rastelli, 2019). Moreover, the complete effect of glyphosate on proteins were not explored. For example, the interactions of glyphosate metabolites such as aminomethylphosphonic acid (AMPA) have been shown to possess binding affinities with certain proteins such as bovine serum albumins, yet they were not explored in this study. (Rajapaksha et al., 2021, John et al., 2023).

Future Implications

To validate the results obtained in this study, it is important to conduct *in vitro* experiments. Isothermal calorimetry can provide the thermodynamic data

on the interactions between glyphosate and the proteins of interest, allowing for comparison with findings from Autodock Tools 1.5.7 Autodock 4.2.6. (Sandoval & Santiago, 2020). To clarify, in isothermal calorimetry, the ligand would be titrated on to a solution of the protein while the heat difference is being measured to calculate the binding energy between the protein and ligand. Moreover, metabolites of glyphosate, like AMPA, should be investigated to understand their interactions with the protein candidates compared to glyphosate. Also, the effect of heavy metals on glyphosate's binding with the target proteins can provide insight into how glyphosate and metal ligands affect protein inhibition or activation. Utilizing model organisms would allow for the introduction of baseline concentration of glyphosate in the bloodstream, enabling the examination of how varying glyphosate levels impact markers for the identified protein targets (uromodulin, prorenin, and interleukin-6).

Developing a regressional or logarithmic model could reveal whether glyphosate affects multiple proteins simultaneously and identify specific serum concentration at which these interactions occur. Understanding this concentration may provide insights into the lethal dosage of glyphosate in model organisms and its potential contribution to CKD.

Also, considering this study's findings that glyphosate may be implicated in both chronic kidney disease and Wilms Tumor, investigating the correlation between glyphosate use and the incidence of Wilms Tumor would be a valuable avenue for future research.

In sum, the results of this study reveal some protein candidates possibly involved in pathways by which glyphosate could affect the diagnosis of CKDu. By shortlisting these possible candidates, focused research studies could be done to understand whether these protein candidates are indeed involved in the diagnosis of CKDu. Additionally, given that the incidence of CKDu is mainly observed across the equator, research groups could further inquire on what geographic, demographic, and environmental factors may further compound with these pathways to increase the relative incidence of CKDu.

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Beyond Existing Analysis: The Rigorous Illumination of Choice from Extant Neuroscience and Philosophy

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Article Synopsis

The age-old question of the existence of human free has been tackled by the field of philosophy traditionally. However, this question has recently been challenged by neuroscience experiments suggesting our decisions are made unconsciously by our brains before we're aware of them. In this article, we explore the validity of this argument and the conclusions it brings regarding human responsibility and morality, and whether this experiment is sufficient to declare the death of human free will as we know it.



Graphic by Chloe Kim

Beyond Existing Analysis: The Rigorous Illumination of Choice from Extant Neuroscience and Philosophy

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INTRODUCTION

Do we have free will? One's first intuition is yes. Humans exercise the freedom of their will incessantly: from deciding this morning's breakfast to choosing a career. And yet, their food decisions could simply be an outcome of chemicals in their brain creating an arbitrary craving, and career choice could be a necessary outcome resulting from a past sequence of events.

These two ideas — neurochemical changes governing decisions and the prior states of the universe determining the current one — form the basis of why many have begun to question our capacity for free will. While we all hold our intuitive understandings and notions as mini-philosophers, free will is generally understood as the capacity of an individual to make decisions based on their desires, independent of external factors. One's actions must not be completely determined by antecedent causes, and in any given situation, one could have chosen to do otherwise (Delnatte et al., 2023).

The problem of whether or not humans possess free will has been a landmark debate of philosophy and theology for millennia, with little progress toward an answer. However, modern technological advances like the fMRI and electroencephalography (EEG) have enabled neuroscientists to gain insight into the decision-making process and, consequently, into the contentious question of whether those decisions are indeed "free" (Delnatte et al., 2023; Dominik et al., 2024).

The most famous of these experimenters, Benjamin Libet, concluded that brain activity precedes the conscious intention to act (B. Libet, 1985). Along

with later work by others involving fMRI and other technology, many groups arrived at a unique conclusion. They reported that human action is decided unconsciously by the brain, and that individuals' consciences are simply made aware of the decision afterward (Brass et al., 2019). This, in conjunction with the contentious scientific standpoint of determinism — which suggests all events are determined *a priori* — has made some conclude that humans do not have free will.

Scientific experimentation and personal experience inform us that the universe flows continually from cause to effect. A physical occurrence, such as an apple falling from a tree, must have some physical reason — or cause — for falling: a gust of wind, an impact from an animal, etc. Generalized, no event can occur which does not have proper causation (Gasparyan, 2024). A natural conclusion is that, given the state of the universe at any past moment, with knowledge of matter and the laws that govern it, one could predict every future or past state of the universe. This includes every action humans have performed or ever will perform. Prior to any "decision", the outcome could be predicted with one hundred percent accuracy. In this view, humans do not choose one course of action over another, we simply act according to the causative forces acting upon us. As a result, many scientists and experimentalists, such as Harvard neuroscientist Robert Sapolsky, have concluded that humans cannot have free will since in any situation we have no capacity to do otherwise (Scott, 2023).

In this paper, we will analyze how neuroscientific interpretations of free will — sparked by Libet and

the scientific notion of determinism — challenge traditional philosophical notions of agency and volition. We will then present a classical style of argumentation known as *reductio ad absurdum*. In this style, one argues that their thesis must be accepted because its rejection would be untenable (Rescher, 2024). A *reductio ad absurdum* argument is a rigorous test for philosophical propositions, which attempts to undermine an argument by following its logic to unreasonable conclusions. In our adaptation of the argument, we explore the conclusions that must naturally follow if we accept many of the beliefs drawn from Libet and determinism. We contend that the absurdity of these claims necessitates a questioning of the premise that brought us to them, namely that we do not have free will. Finally, we present alternative interpretations of Libet's experiment and what contemporary neuroscientific findings have to offer on the topic.

We argue that the evidence against mankind's possession of free will is unreasonable given its implausible logical follow-through and unsound evidential basis. Based on this, we infer that neuroscience is not yet sufficiently equipped to contend with such fundamental questions as the human possession of free will.

BACKGROUND

Aristotle sufficiently paints one of the first strokes of the Western understanding of free will in *Nicomachean Ethics* by discussing the ability of human choice and action, and how “the man is the father of his actions as of children” (O'Connor & Franklin, 2022).

In a more modern context, almost every notable philosopher of the twentieth century has given their perspective on the issue of free will. In this debate, philosophers widely shared two assumptions: the notion that moral action cannot exist without free will and that our notions about free will seem difficult to reconcile with the world. Following the second issue, groups had a wide range of concerns. For some, it was theological: how do you reconcile freedom in a world of an ultimate and all-powerful creator? For some like Descartes, it was a strictly scientific issue: how can free will arise from the physical framework of a world

which is deterministic? The notion of determinism, which dictates the predictability of the universe, has a significant foothold in the issue of free will, and can strongly inform us about its existence, or lack thereof (O'Connor & Franklin, 2022).

While traditional philosophy's approaches and insights into the issue of free will have resulted in wide disagreement within the field, these debates have given rise two primary claims that can be used to study and discuss this problem. The first of these claims is that “free will has two aspects: the freedom to do otherwise and the power of self-determination” (O'Connor & Franklin, 2022). This notion of “freedom to do otherwise” is studied as volition, while agency deals with the power of self-determination (Haggard, 2019). The other claim states that adequate accounts of free will must entail free agents that are morally responsible agents (O'Connor & Franklin, 2022). In order to be held responsible for an action, one must have been freely able to choose for, or against, that action. In this paper, we will follow and use these foundations as groundwork to study free will. We will use and define the issue of free will through the lens of volition and agency, and study free will, or the lack thereof, with the idea of social and personal moral responsibility.

Through this lens, classical philosophers came to a variety of conclusions. Thomas Reid was a proponent for free will, believing that humans have the agency to make choices beyond their simple desires. Hobbes, among others, have argued for the existence of a free will which is compatible with determinism, which instead relies on a free will defined by a lack of restriction by external factors rather than a control which goes beyond ones will. In this view, free will is obtained when one can follow their will without restriction (O'Connor & Franklin, 2022). Nietzsche and others have argued the simple impossibility of free will to exist, an argument much more radical than saying humans are agents lacking control (Gemes, 2009). In this view, there is no physical realm in which free will could evolve, while the latter claim suggests that for a multitude of reasons (social, psychological, biological) the exercise of free will is illusory. Despite centuries of work on this topic, no verdict has yet been reached.

LIBET'S EXPERIMENT

Among the many who have approached the problem of free will, few have gained traction like Libet's famous experiment in 1983 (B. Libet, 1985). In this section, we discuss the setup and findings of this experiment in order to analyze its implications on free will in the following section.

In this experiment, Libet had participants carry out a simple action, such as flexing their wrist or pushing a button, at a self-determined time. As they did this, they watched a clock face that hosted a revolving dot and were tasked with self-reporting the dot's position on the dial when they became aware of their intention to perform an action. An electroencephalograph recorded neuronal activity while the participants performed their tasks. In this experiment, the moment an individual becomes aware of their intention to act was referred to as "W" (Gasparyan, 2024).

The purpose of this setup is to expand research on a previously discovered wave of brain activity called readiness potential (RP) which was found to precede voluntary movement. Libet observed that the onset of RP occurred an average of 350-400 ms prior to W (Brass et al., 2019; Gasparyan, 2024; B. Libet, 1985). This particular pattern of brain activity could routinely be observed before individuals reported forming their intention to act.

From this discrepancy in timing, Libet made his most important and influential conclusion. Firstly, RP onset can be reliably used to predict W. The RP brainwave appeared to always precede conscious intention formation. From this, Libet argued that the conscious experience of decision-making occurs only after a duration of brain activity which shows action preparation. Conscious decision-making follows significant brain activity. Hence, the process of decision-making is initiated unconsciously. In Libet's own words, 'the brain "decides" to initiate or, at least, to prepare to initiate the act before there is any reportable subjective awareness that such a decision has taken place' (B. Libet, 1985). That is, when we make a decision, our brain has already begun acting before we're conscious of it. Having gathered the setup, we explore the outcomes of Libet's Experiment.

IMPLICATIONS OF LIBET'S EXPERIMENT ON FREE WILL

According to Libet, the volitional process, or the sequence of events which lead to a decision, is initiated unconsciously as W occurs 350–400 ms after RP starts, but 200 ms before the motor act (B. W. Libet, 1999). This experiment had massive implications and produced intense debate in the field. Some psychologists surveyed his findings, and combining them with their work in the field, argued that the experience of conscious willing is 'an illusion' (Wegner, 2002). Other authors have replicated this finding in a variety of experimental setups to counter experimental biases. Notably, Matsushashi and Hallett replicated Libet's findings without relying on subjective reports or clock memorization on the part of participants (Matsushashi & Hallett, 2008). All these findings were interpreted by Sapolsky, who felt that if these findings and others from adjoining disciplines were combined, there would be "no room for free will" (Scott, 2023).

What one can conclude is that Libet's work and its derivative experiments suggest a neuro-philosophical framework that implies that human actions do not arise consciously, a conclusion which would be consistent with a philosophical framework indicating a lack of free will. This conclusion, however, can lead to some interesting perspectives on our current social order, which may be unreasonable.

REDUCTIO AD ABSURDUM

In this section, we analyze these perspectives on our social order, employing the *reductio ad absurdum* style of argumentation, in which we posit that the implications of a lack of free will are absurd and contrary to reason, thus one must reexamine the premise which led us to them, specifically, the premise that we do not have free will.

One of the primary conclusions that arises from the lack of free will is that the concept of culpability, as in law, has no basis. Culpability is the foundation of legal sentencing, and it is the extent to which an individual is blameworthy, or in other words, responsible, for their actions. Responsibility is a function of two things, one of which is the ability

of an individual to conform their actions to the law (Steinberg, 2013). However, in this Libet-inspired view held by individuals such as Sapolsky, a person's subconscious dictates their actions, and . For a criminal, this means there was no exercise of free will in performing any unlawful acts. We are forced to conclude then that any individual on trial cannot conform their actions to the law, and cannot be considered at fault for their actions (Helfrich, 2023).

In light of this, some holders of this view argue for a shift from criminal punishment to criminal rehabilitation, yet the fundamental hypocrisy inherent in this stance is evident. The goal of these groups relies on a willful action by an individual in power to enact change, yet the foundation of their view is that free will does not exist.

In light of this, some holders argue for a shift from criminal punishment to criminal rehabilitation, however even this movement seems weakened by its foundational beliefs. It is difficult to rationalize changing the mind of any individual or group in a world with no free will. After all even if they disagree, by the very premises on which the argument stands, they cannot be held at fault.

Let us look at another foundational aspect of society: the recognition of certain actions as "good" and others as "bad", "right" and "wrong". Racism in America and the recognition of its inhumanity, its universally condemned depravity, sparked the Civil Rights movement, and the heinous actions of Hitler during WWII induced the United States to join in the war against him. But once again, if the actions of those in power were the only ones possible, not chosen as an act of their free will, then these movements have no ground on which to stand. The United States could have had no other possible social structure at the time, and if we now incorporate a deterministic worldview, Hitler's goal of Jewish extermination was simply the present manifestation of a prior state of the universe. In the same way that a rock cannot be judged for its shape forged by winds and erosion over time, humans cannot be judged for their actions. "Good" and "bad" do not exist in this context. There simply "is" (Gasparyan, 2024). Inequality becomes no better than equality, and genocide becomes as natural as peace:

in a purely deterministic universe, our attributions of moral value to different social conditions would be arbitrary classifications imposed on what are equally necessary outcomes of physical law.

Lastly, let us look at another pillar of modern society, the social contract. This is the "contract" an individual forms with their government which dictates that the individual will give up some rights in order that the government may in turn provide services to the individual and protect their remaining rights (D'Agostino et al., 2024). It is a voluntary ceding of rights, and this transaction is the foundation of governments around the world. It is clear by now that any "voluntary" action is not truly voluntary. No exercise of will can be used to volunteer one's rights because no exercise of will exists. One cannot truly choose to enter or leave such a contract if all decisions are predetermined. This shift in perspective implies not that the social contract lacks benefits, but that it loses its depth and meaning. For example, patriotism itself becomes difficult to justify in this framework, as one's attachment to their country would become a result of predetermined factors rather than a conscious choice. Thus, mankind must renounce another fundamental principle if the current scientific view is to be accepted. In this framework of belief, the nature of reality becomes difficult to come to terms with.

So, let us for a moment say that neuroscience appears on solid ground to indicate that our actions are not the result of our conscious intentions but rather the unconscious workings of our brains, and science as a whole would indicate that due to determinism, no other course of events could have transpired than those which did. Yet, if we follow the natural course of this logic, we arrive at absurdity: law has no basis; there is no right or wrong; a fundamental aspect of the relationship between the government and the governed has been shaken. Those things which humans feel to be most self-evidently true are suddenly undermined.

As a result, we argue not that these conclusions should be accepted, but conversely, that the conclusions drawn from the natural extension of Libet's findings and the idea of determinism itself are

so absurd that they cast doubt on the validity of the presuppositions that led us to them. It is more likely that the arguments derived from Libet's experiment are erroneous, and that perhaps we do not live in a deterministic universe, than for actions to be valueless and for free will to be but an illusion.

FURTHER NEUROSCIENTIFIC PERSPECTIVES

Apart from the unsettling social conclusions that arise from rejecting notions of free will, most new neuroscientific accounts seem hesitant to declare the verdict of a lack of free will. It is relevant to recognize that to prove a case for the reality of free will, we only need to prove our worth as active agents of our actions. That is, humans must be the fundamental source of their actions (O'Connor & Franklin, 2022). However, to disprove free will, one must also prove a case for causal determinism, which says prior conditions fully determine human behavior.

In the case of causal determinism, then, most who wish to declare the death of free will fail to give sufficient evidence through Libet's Experiment, because it cannot fully explain the mechanisms behind the free decision-making process and outcome. Notably, Libet, in his later work, remarks that conscious will affects the outcome of the volitional process even though unconscious processes initiated it (B. W. Libet, 1999). He hypothesizes that consciousness serves as a trigger required to enable the volitional process to proceed to final action, even if the process is initiated unconsciously. This creates a framework where lower-order workings of the brain can be consistent with the higher-order "conscious" thought that free will suggests. We need to recognize that brain activity in Libet's Experiment reflects the decision-making process not the outcome of it (Brass et al., 2019).

Some novel insights into the decision-making process have arisen from the "free-choice versus stimulus-driven action" paradigm (Delnatte et al., 2023). Freely-willed actions are internally generated, unlike stimulus-driven behaviors. For example, choosing to go for a walk stems from internal motivations, while obeying a traffic signal is externally triggered. Free will entails decision-making: choosing what,

when, and whether to act. In experiments analyzing this paradigm, participants freely select actions, timing, or whether to act, contrasting with stimulus-driven scenarios where actions are dictated (Brass & Haggard, 2008; Fried et al., 2017; Seghezzi et al., 2019). Neuroscientists believe that characterizing these mechanisms should provide further insight into the question of determinism and free will.

Through several such experiments using fMRIs, electrophysiology and lesion studies, scientists have come to a variety of conclusions about the nature of decision-making: Some believe that the accumulation of stochastic neural noise makes the signal cross the decision threshold for neurons to fire, while others attribute this to an accumulation of contextual evidence (Bode et al., 2014; Brass et al., 2019). Stochastic noise refers to truly random fluctuations in neural activity, rather than neural activity which is deterministically elicited. This suggests that a cause cannot always be pointed to as fully explanatory of our actions, which may leave room for free will. Contextual evidence, however, involves recent choice history. This implies a deterministic front on free choice as past actions may influence present actions, but not fully so because factors like stochastic noise still exist.

These conclusions, among many others, lead us to degrees on the spectrum of free will, but none can justify a lack of will. Some scientists voice these concerns, remarking how neuroscience can at most "highlight the factors that can push our choices in one or another direction", but not declare the verdict on free will (Nichelli & Grafman, 2024).

One thing that has remained consistent in this discussion, however, is that the experimental setup used to investigate free will in Libet's experiment has its limitations— and they are significant. While some of these concerns were the product of research design, like issues inherent in clock reading and self-reporting biases in Libet's original experiment, and could be resolved by alterations, other concerns were much deeper. Recent research has found readiness potentials to be absent for deliberate decisions and preceded arbitrary decisions only (Maoz et al., 2019). Others associate RP not only with conscious action but also

to unconscious choices and expectations (Dominik et al., 2024). In this sense, we are still uncovering more about this single experiment alone.

Even beyond Libet, current experimentation and bodies of work focus extensively on decision-making processes and lower-level causation, and cannot sufficiently evaluate the issue at hand. It seems that, even though neuroscience has seen evidence from both sides, the case of free will remains open.

CONCLUSION

The debate over whether humans have free will - the capacity of an individual to make decisions based on their desires independent of external factors - has raged for millennia with little progress. Recently, however, neuroscience has achieved the ability to empirically broach the subject. The most notable findings come from Libet's experiments and its derivatives, which indicate that free will is merely an illusion; our actions are the result of unconscious brain activity rather than conscious intention. This has become a standard belief among the scientific community in recent times due to its empirical basis in conjunction with deterministic theory.

However, if we accept this conclusion, then we must reject ideas of right and wrong, good and bad. We must throw away the belief that anyone can be guilty of a crime and in the same stroke sweep away the basis of a government's relation with its people. As a result, we argue that the absurdity of these conclusions necessitates questioning the premise that brought us to them, namely that we do not have free will. Furthermore, doubt in the reliability of conclusions drawn from Libet's experiment is validated by more recent neuroscientific analyses of his experiments and those like it, which concur with various compatibilist views.

We believe that the conclusion that humans do not have free will is not reasonable given its outlandish logical reasoning and unsound evidential basis. Noting the many developments happening in the field, we acknowledge that while neuroscience is not yet well equipped to contend with such fundamental questions as the human possession of free will, there may be more conclusive answers in

the future.

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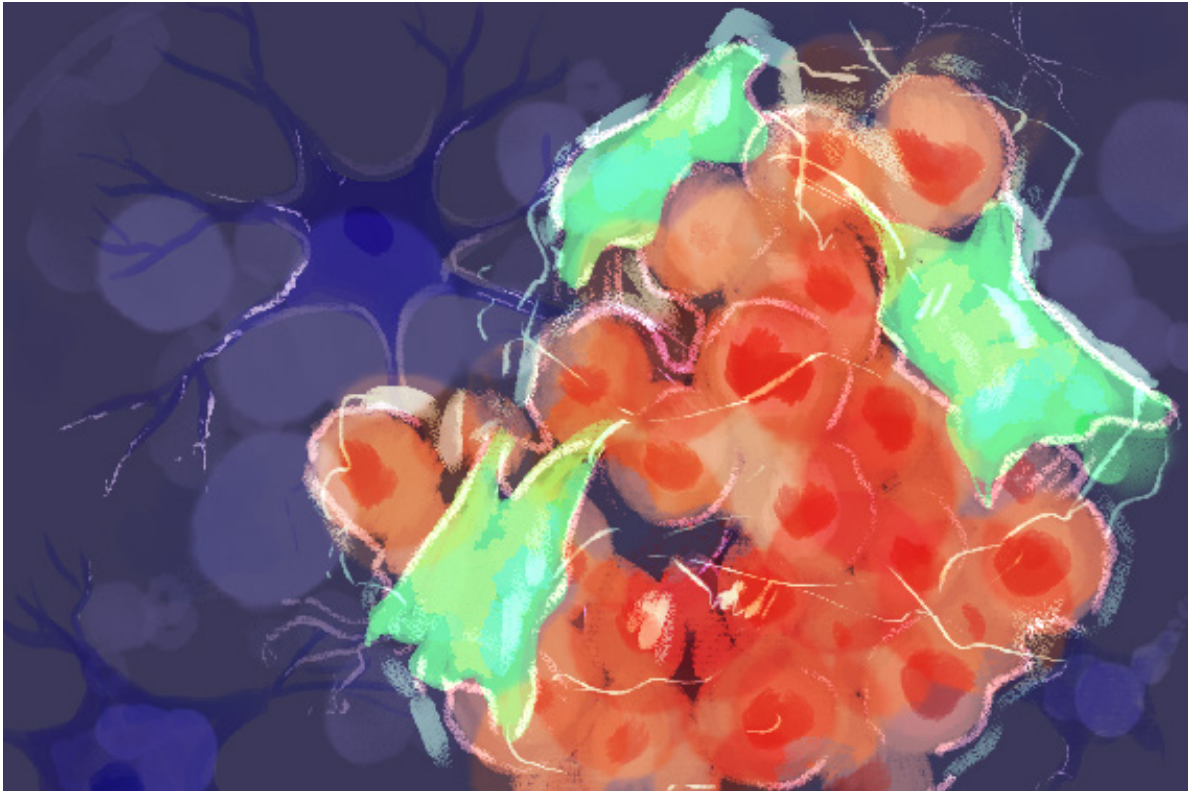
The Influence of Cancer Associated Fibroblasts on Glioblastoma Tumor Migration

Eugene Cho



Article Synopsis

Glioblastoma is a complex cancer that resides in an intricate tumor environment in the brain. Cancer associated fibroblasts have previously shown to contribute to tumor expansion, and similar cells have been found in the brain of Glioblastoma patients. This study demonstrates the influence of cancer associated fibroblasts on tumor cell migration which may contribute to patient outcomes.



Graphic by Amanda Li

The Influence of Cancer Associated Fibroblasts on Glioblastoma Tumor Migration

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ABSTRACT

Glioblastoma (GBM) is a malignant central nervous system tumor with a low patient prognosis. Given this challenging outlook, there has been a great interest in the development of immunotherapies for patients with GBM. To enhance the efficacy of immunotherapy, researchers have studied the immunosuppressive nature of the GBM tumor microenvironment (TME). One element of the TME that is of particular interest is cancer associated fibroblasts (CAFs). Often found in carcinomas, previous studies of single cell RNA sequencing have revealed CAF-like cells in the GBM TME, which promotes angiogenesis and hinder immune cell infiltration. This study revealed that GBM tumor cells and GBM CAFs cannot be easily differentiated by the following surface markers: CD45, CD73, CD90, CD49e, CD105, CD140b, and CD29. Additionally, the presence of GBM CAFs was found to induce GBM tumor cell migration and the addition of IL-12 to the GBM CAFs was found to reduce GBM tumor cell migration. This project aimed to understand the differences in surface markers of GBM tumor cells and GBM CAFs, as well as to explore the influence of GBM CAFs and the cytokine IL-12 on GBM tumor cell migration. This research can supplement existing efforts to increase the efficacy of immunotherapies when trying to target GBM.

INTRODUCTION

Glioblastoma (GBM) is the most common malignant tumor of the central nervous system with a median prognosis of about 15 months (Weenink et al., 2020). Many efforts have been made to treat patients with GBM through chemotherapy and radiation; however, there is no standardized method of treatment proven to be effective for all GBM patients (Ostrom et al., 2017). The heterogeneity of GBM contributes to difficulties in treating patients at the population level. Therefore, immunotherapy has been widely explored as a way to treat GBM, by taking advantage of patient-specific characteristics and recruiting a patient's own immune cells to target the tumor (Weenink et al., 2020). By activating T-cells and other immune cells, their ability to infiltrate and target tumors can be further optimized to improve patient prognosis.

Tumor Microenvironment

To study the efficacy and optimization of immune

cell infiltration in GBM, elements in the tumor's microenvironment must be considered. Although immune cells are able to cross the blood-brain barrier and target GBM tumor cells, its immunosuppressive tumor microenvironment (TME) reduces the efficacy of immune cell infiltration (Genoud & Migliorini, 2021). In other words, immune cells are easily exhausted in the GBM TME. The TME is made up of cancer cells, brain cells, and immune cells, including microglia, natural killer (NK) cells, and endothelial cells (Sharma et al., 2023). Elements in the tumor microenvironment communicate with other immune cells through the release of cytokines and chemokines. These soluble factors can be involved in both tumor progression and suppression (Abdul-Rahman et al., 2024). Specifically, the cytokine interleukin 12 (IL-12) has been essential in directing T-cells into the tumor microenvironment and exhibit antitumor properties (Abdul-Rahman et al., 2024). IL-12 has been of great interest in immunotherapies

with its abilities to increase the persistence of T-cells, despite its toxicity in clinical studies (Agliardi et al., 2021). It will be important to explore the additive effect of IL-12 on GBM tumor cell migration.

Cancer Associated Fibroblasts

One element of the TME that is of particular interest is cancer-associated fibroblasts (CAFs). CAFs have been detected in carcinomas, like breast cancer and lung cancer, where they influence the surrounding tumor environment. They contribute to angiogenesis, meaning the growth of new blood vessels, encourage tumor expansion, and reduce the efficacy of immunotherapies (Galbo et al., 2022; Zarodniuk et al., 2023). Additionally, CAFs are largely responsible for remodeling and altering the extracellular matrix (ECM) (Sahai et al., 2020). This remodeling can make it even more challenging for immune cell infiltration and the killing of tumor cells.

Although fibroblasts are not found in the brain, studies have shown the presence of CAF-like cells or mesenchymal stem cells (MSCs) that share characteristics with CAFs found in carcinomas (Jain et al., 2021). Using single-cell sequencing, researchers have confirmed the presence of CAF-like cells in the GBM TME. Furthermore, single cell RNA sequencing has revealed shared characteristics between CAFs and GBM CAFs, like shared surface markers and their role in tumor progression (Galbo et al., 2022; Jain et al., 2021). Some shared surface markers include fibroblast-specific protein 1 (FSP1), alpha-smooth muscle actin (α SMA), fibroblast activation protein (FAP), PDGFRA, and PDGFRB (Galbo et al., 2022; Zarodniuk et al., 2023). Further, studies have identified surface markers on CAFs, to differentiate it from other cell types in the TME. CAFs were found to express CD73, which is involved in tumor adhesion, proliferation, and chemoresistance and CD140b (PDGFRb), a CAF marker known to be important in CAF formation and promote angiogenesis (Jain et al., 2021; Yan et al., 2019). Additionally, CD29 is integrin B1 and is found to be characteristic of CAFs in breast cancer. Lastly CD49e is integrin α 5 that binds to fibronectin, which mediates the relationship between CAFs and cancer cells (Galbo et al., 2022; Han et al., 2020; Pelon et al.,

2020).

GBM CAFs are of interest due to their ability to promote angiogenesis, modify the GBM extracellular matrix (ECM), and create a physical barrier to infiltrating immune cells in the brain (Galbo et al., 2022). More specifically, the abundance of GBM CAFs can alter the ECM, promoting GBM migration (Zarodniuk et al., 2023). GBM patients were found to have greater tissue remodeling compared to patients with low-grade gliomas. Further, patients with a greater abundance of perivascular fibroblasts experienced poorer prognosis and survived for a shorter period of time (Zarodniuk et al., 2023). Consequently, this finding suggests that the presence of increased fibroblasts contributes to the immunosuppressive TME, resulting in a reduced immune response. Other studies have demonstrated that fibroblasts serve as a chemical and physical barrier against immune cells by secreting cytokines and exhausting and reducing the efficacy of immune cells that try to infiltrate (Hilmi et al., 2020).

Current Study

This project aims to explore the differences between tumor cells and GBM CAFs and to determine the influence of GBM CAFs on GBM tumor cell migration using flow cytometry and transwell migration assays. Additionally, the project aims to determine the role of IL-12 on GBM migration when added to GBM CAFs. It is hypothesized that GBM tumor cells and GBM CAFs can be differentiated by the following surface and intracellular markers: CD45, CD73, CD140b, GFAP, CD29, and CD49e. It is also hypothesized that the presence of GBM CAFs will promote GBM cell migration; however, the addition of IL-12 to GBM CAFs will reduce GBM tumor cell migration. This project will add to the existing knowledge about the effects of the immunosuppressive GBM TME by providing insight about CAF characteristics and its role on GBM tumor cells.

METHODS

Cell Culture

The U87 GBM mouse cell line was cultured in Dulbecco's Modified Eagle Media (DMEM) and 10% Fetal Bovine Serum (FBS). The U87 cells were thawed

into a T75 flask and once 80-90% confluent (about 2 days after), the cells were passaged with 0.05% Trypsin ethylenediaminetetractic acid (ETDA), centrifuged, counted, and split based on their cell counts. The cells were then frozen down at passage 8 when about 90 million cells were cultured.

The GBM CAFs (Vitro Biopharma, Cat. No. CAF03) were cultured in VitroPlusIII Low Serum, Complete Medium (Vitro Biopharma, Cat. No. PC00B1). The GBM CAFs were thawed in a T150 flask and split once 80-90% confluent (7 days) as per supplier protocol.

Identification of Surface Markers through Flow Cytometry

Flow cytometry is a method of identifying the shape and size of cells, as well as intracellular and extracellular markers present on the cells. The flow cytometry panel was developed using surface and intracellular markers found in the GBM CAFs based on existing literature. Additionally, the fluorescent colors of antibodies were determined by comparing the excitation wavelengths of the different antibodies of interest. When compared to the fluorescent expression of a one cell to that of a second cell, a shift in the expression of the second cell would indicate a difference in the expression of the surface marker of interest between the two cells. Whereas if there is not a difference in the shift in the two cells, that would indicate no difference. The GBM CAFs and U87 cells were stained for the antibodies and prepared for flow (Table 1).

Table 1: Flow panel for CAF markers of interest

	Marker	Laser Line	Color	Location
1	Live/Dead	UV6	Zombie UV	
2	CD45	UV16	Buv805	Extracellular
3	CD73	V3	PacBlue	Extracellular
4	CD90	V10	BV605	Extracellular
5	GFAP	B2	AF-488	Intracellular
6	CD49e	YG1	PE	Extracellular
6	CD105	YG9	PE-Cy7	Extracellular
8	CD140b	R1	APC	Extracellular
9	CD29	R7	APC-Cy7	Extracellular

Transwell Migration Assay

While GBM CAFs were initially cultured in VitroPlusIII Low Serum, Complete Medium, the GBM CAFs were cultured in DMEM with 10% FBS for the transwell migration assay. By splitting the GBM CAFs into three flasks containing VitroPlusIII Low Serum, Complete Medium, DMEM/10% FBS and Advanced DMEM/F12, it was determined that DMEM/10% FBS promotes the growth of the GBM CAFs and therefore can be used for the transwell migration assay. Transwell assays are important in assessing how cells respond to certain chemo-attractants (like cytokiines) of interest. The transwell plate (Thermo Scientific™, Cat. No. 141006) had an 8µM filter, with an approximate distance of 1.0mm from the insert to the bottom membrane.

GBM CAF-conditioned media (CM) was prepared by seeding 25,000 CAFs into the number of wells needed for transwell migration towards CAF CM in 10% FBS. Following 24 hours, the media was removed and the CAFs were washed with phosphate-buffered saline (PBS). Serum-free media was added to the wells and left to culture. After 72 hours, the media was collected and immediately used for transwell migration.

The transwell migration assay included 9 groups for testing, which were arranged onto three 24-well plates (Table 2 & Table 3). The top insert contained the GBM tumor cells, and the bottom insert contained GBM CAFs, GBM CAFs supplemented with IL-12, CAF CM, or CAF CM supplemented with IL-12. This transwell was designed to test the migration of GBM tumor cells downward towards the GBM CAFs and CAF CM.

Table 2 Transwell migration conditions

Well	Top Insert	Bottom of Well	Justification
1	Empty	CAF cells	CAF control for counting/flow
2	Empty	U87	Control for 100% migration
3	Empty	U87 & CAF CM	Control for 100% migration in soluble factors
4	U87	DMEM/2% FBS	Negative Control
5	U87	DMEM/30% FBS	Positive Control
6	U87	CAF cells	Migration towards CAF cells
7	U87	CAF CM	Migration toward CAF soluble factors
8	U87	CAF & IL-12	IL-12 and CAF induced migration
9	U87	CAF CM & IL-12	IL-12 and CAF soluble factors induced migration

Table 3: Layout of three 24 well plates used for transwell migration.

1A	1B	1C	1D	1E	1F	4A	4B	4C	4D	4E	4F	6A	6B	6C	6D	6E	6F
2A	2B	2C	2D	2E	2F	5A	5B	5C	5D	5E	5F	7A	7B	7C	7D	7E	7F
3A	3B	3C	3D	3E	3F							8A	8B	8C	8D	8E	8F
												9A	9B	9C	9D	9E	9F

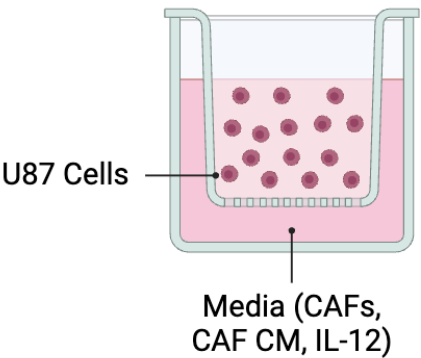


Figure 1: Layout of transwell migration insert with U87 tumor cells in the top insert and CAF media on the bottom.

Following the transwell assay, the U87 tumor cells and GBM CAFs were dyed using membrane dye to differentiate between the two cell types. GBM CAFs were thawed in a T150 flask and dyed with CellTracker Green CMFDA, then 25,000 GBM CAFs were seeded into the bottom chamber of the transwell. The following day, the U87 tumor cells were dyed using CellTrace Red and 100,000 U87 cells were seeded into the insert

or bottom of the transwell. Then the U87 cells were left to migrate for 24 and 72 hours (Figure 1). Fluorescent images of the cells were taken (Figure 4). Afterwards, the cells were fixed and prepared for analysis using flow cytometry.

RESULTS

Differentiation of CAFs and GBM CAFs through Flow Cytometry

Upon running flow cytometry on the GBM CAFs and U87 cells, differentiation of the two cell types was not possible. Although there were differences in the expression of some surface markers, the expression of other surface markers was too similar between the U87 cells and GBM CAFs. There were differences in expressions of CD45, CD73, and CD140b, which were demonstrated by the shift in red peak (stained U87 cells) from the stained blue peak (stained GBM CAFs) (Figure 2). However, there were no differences in expression of GFAP (glial fibrillary acidic protein), CD29, and CD49e (Figure 3). The overlap in GBM CAF and U87 peaks demonstrate similar expression

of the surface markers. Therefore, the U87 cells and GBM CAFs could not be confidently differentiated by these surface markers.

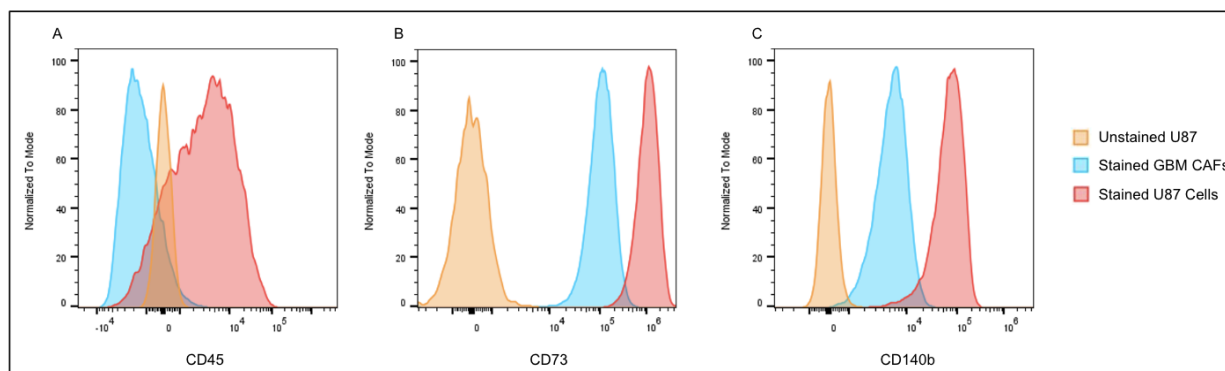


Figure 2: Flow histogram demonstrating that U87 cells and GBM CAFs can be differentiated by A) CD45 B) CD73 C) CD140b expression.

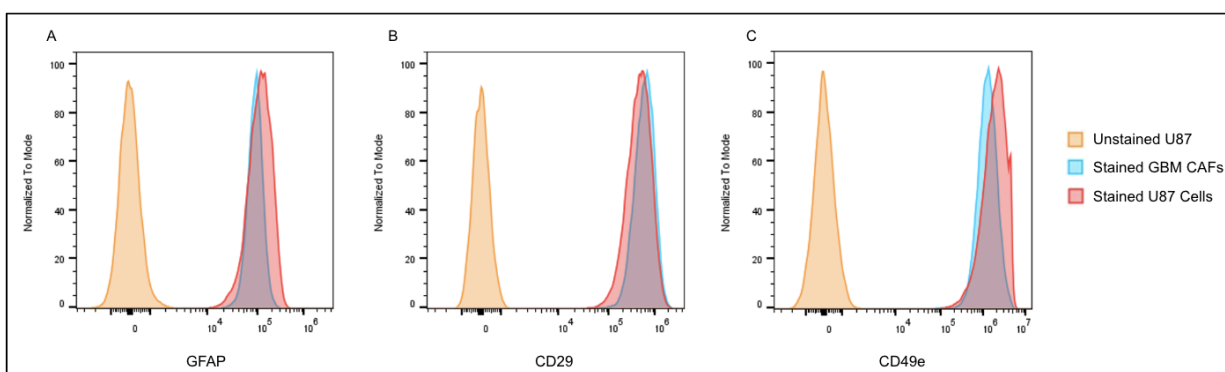


Figure 3: Flow histogram demonstrating that U87 cells and GBM CAFs cannot be differentiated by A) GFAP B) CD29 C) CD49e expression.

GBM CAF Transwell Migration Assay

Transwell migration assays allowed for the exploration of U87 tumor cell migration in response to the presence of CAFs. The U87 tumor cells were seeded in the transwell migration wells in different conditions. Figure 4 presents the CAF cells (Fig. 4A), U87 cells in DMEM (Fig. 4B), and U87 cells in CAF CM (Fig. 4C), seeded at the bottom of the inserts, showing the maximum number of cells that would be present given full migration.

While 100,000 U87 cells were seeded at the bottom of the wells prior to migration, the U87 cells were determined to have roughly doubled during the 72-hour migration period (Figure 5). In DMEM supplemented with 2% FBS, the U87 cell count

increased, on average, to 220,220 cells, while in the CAF-conditioned media (CM), the U87 cell count increased to 226,480, on average. This difference was not statistically significant, highlighting the potential role that CAFs have in releasing cytokines that can be involved in CAF proliferation.

Additionally, migration of the U87 cells through the transwell inserts, with base media supplemented with varying amounts of FBS, was determined by the positive and negative controls (DMEM supplemented with 30% FBS and DMEM supplemented with 2% FBS respectively) (Figure 6). Based on the fluorescent image alone, differences in U87 migration in the two control groups were not identifiable. However, upon running the samples on flow, there were, on average, 23 and 61 cells for the negative and positive controls

respectively (Figure 6C).

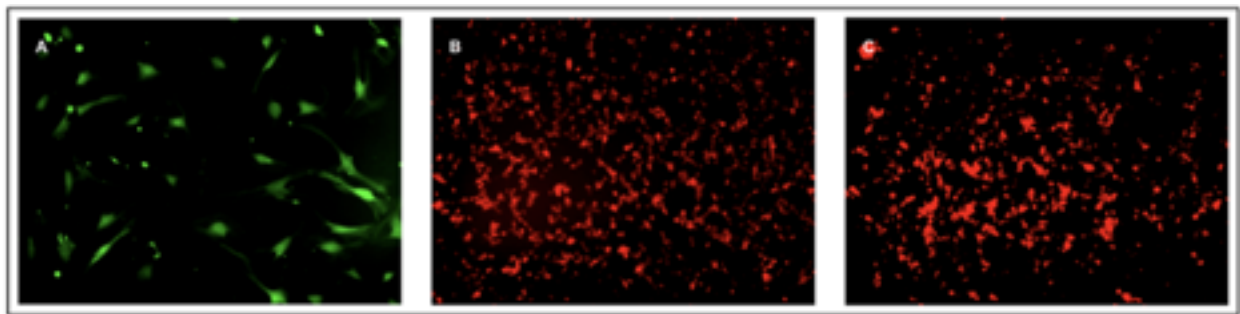


Figure 4: Fluorescent images of (A) CAF cells plated at the bottom of the well (B) U87 tumor cells plated at the bottom of the well in DMEM/2% FBS (C) U87 tumor cells plated at the bottom of the well in CAF-conditioned media.

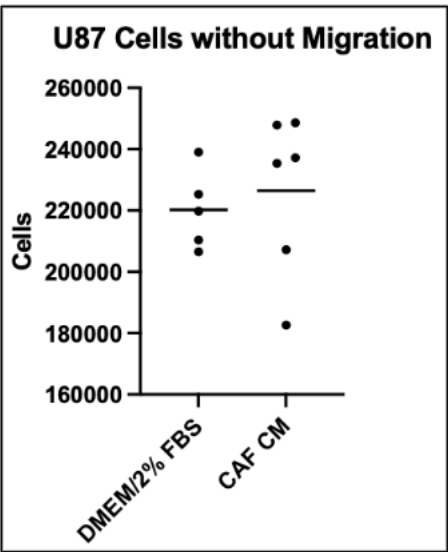


Figure 5: U87 cell count without migration following 72 hours.

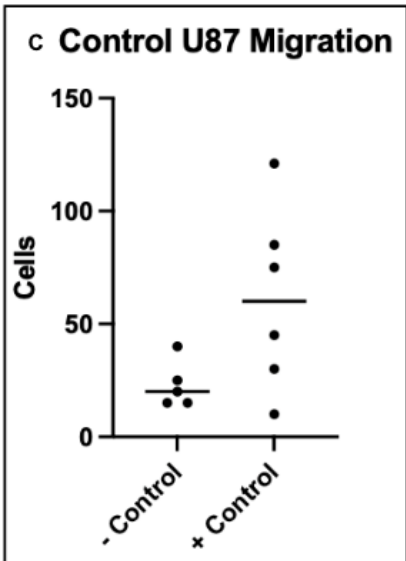
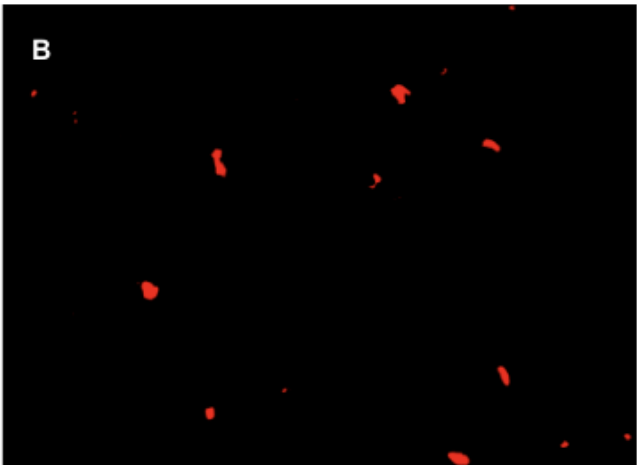
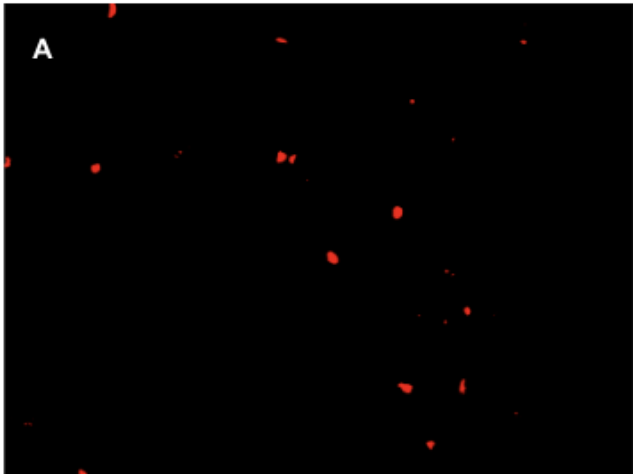


Figure 6: Fluorescent images of A) Negative control of U87 cell migration to DMEM/2% FBS (minimal growth factor) B) Positive control of U87 cell migration to DMEM/30% FBS. C) Cell counts of U87 cell migration in the positive and negative controls.



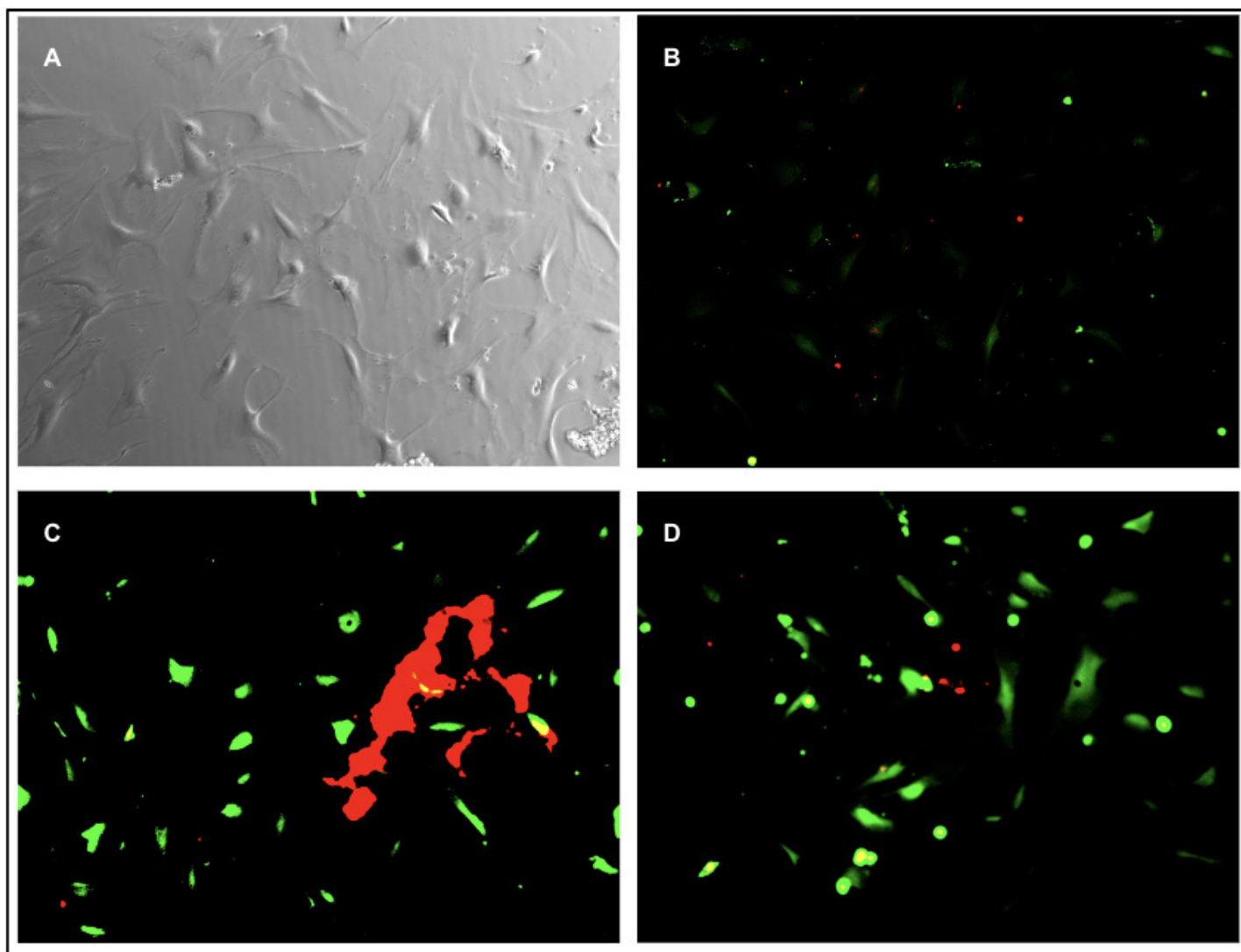


Figure 7: A) Brightfield image of U87 cell co-culture with CAF cells and B) Fluorescent image of U87 cells (red) that have migrated towards the CAF cells (green) C) U87 cells that have migrated towards CAF cells that appear to be clumped together C) U87 cells that have migrated towards the GBM CAFs supplemented with IL-12.

Fluorescent imaging shows U87 cells that have migrated towards the CAFs following a 72-hour migration. Visually, it is difficult to determine the extent to which the U87 cells have migrated towards the CAFs (Figure 7). In some instances, the U87 cells seem dead, forming clumps when co-cultured with the GBM CAFs (Figure 7c).

The number of U87 cells that have migrated towards the GBM CAFs was also quantified. More U87 cells migrated towards the GBM CAFs in 72 hours, than in 24 hours (Figure 8). On average, 23 U87 cells migrated towards GBM CAFs in 24 hours, while 121 U87 cells

migrated towards GBM cells in 72 hours. 50,000 U87 cells were seeded in the top insert of the well, prior to the 24-hour migration period, while 100,000 U87 cells were seeded in the top insert prior to the 72-hour migration. Figure 8 demonstrates the percentage of U87 migration after 24 and 72 hours to demonstrate the percentage difference in migration. Following the 24-hour migration period, on average, 0.047% of U87 cells migrated towards the GBM CAFs, while following a 72-hour migration period, 0.121% of U87 cells migrated towards the GBM CAFs (Figures 8C and 8D).

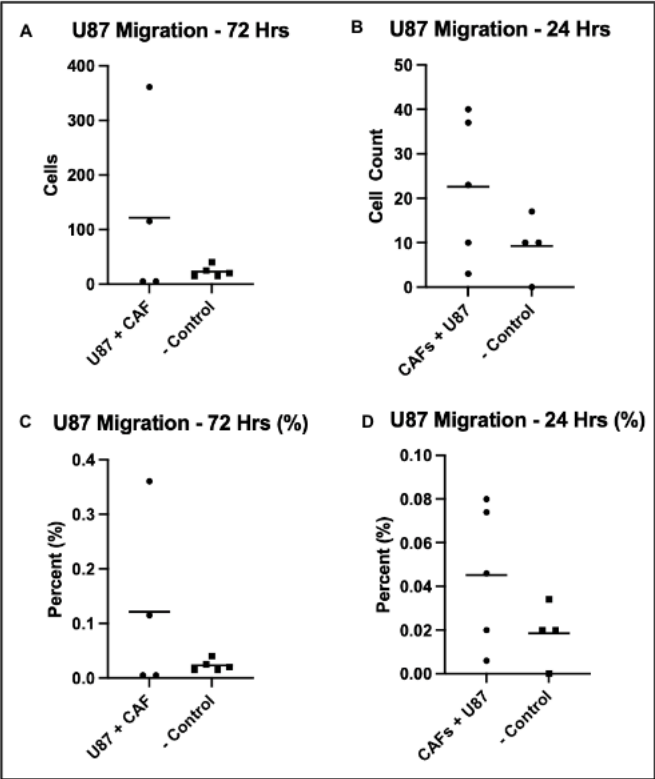


Figure 8: Number of U87 cells that have migrated following A) 72-hour migration period B) 24-hour migration period. Percentage of U87 cells that have migrated following C) 72-hour migration period D) 24-hour migration period.

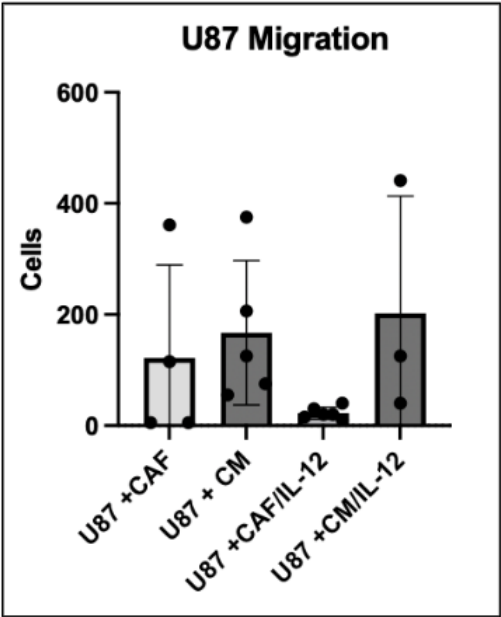


Figure 9: U87 cell migration towards GBM CAFs, GBM CAF Conditioned Media, GBM CAFs supplemented with IL-12 and GBM CAF Conditioned Media supplemented with IL-12.

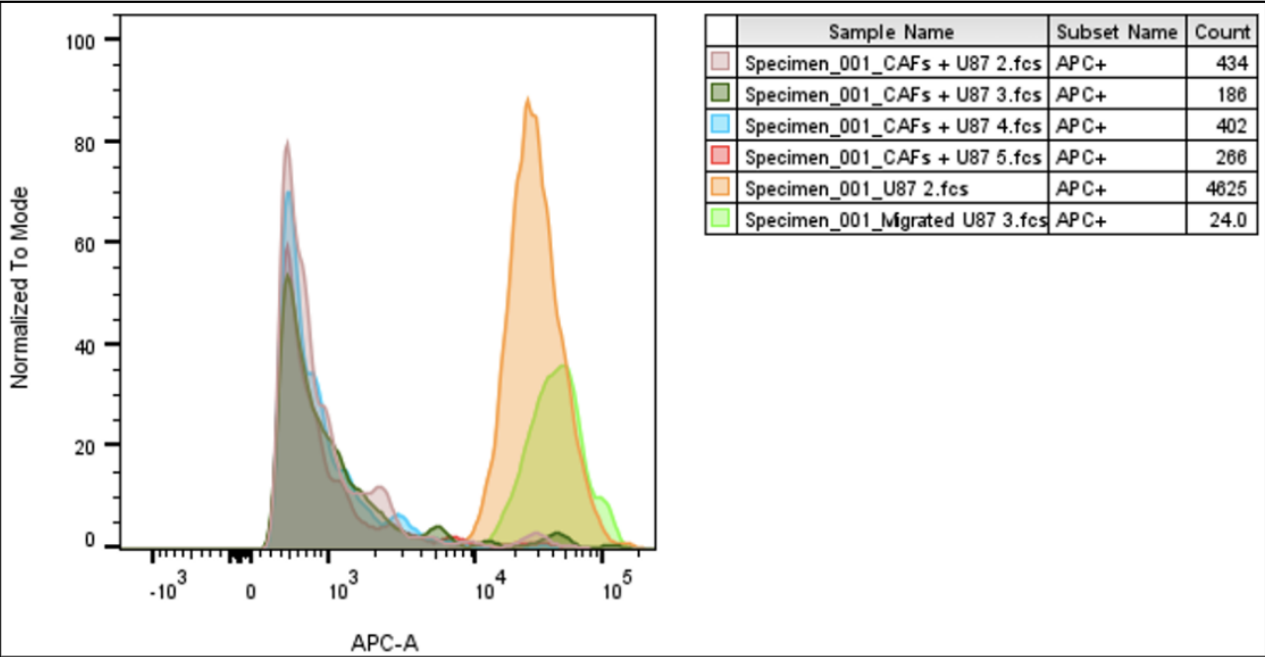


Figure 10: Proliferation of U87 cells can be seen by the dimming of the APC expression by cells on the left when co-cultured with the CAFs following migration.

Another observation was the proliferation of the U87 tumor cells when co-cultured with CAF cells. Following the initial 24-hour migration assay, the U87 cells exhibited diminishing expression of allophycocyanin (APC), a fluorescent protein that can stain cells of interest, which led to the hypothesis that the U87 could be proliferating upon interaction with the GBM CAF soluble factors. If the cells were proliferating, it would be expected that upon each division, the cell would express reduced levels of APC as shown in figure 10. The U87 cells that migrated in the control conditions and that were cultured alone exhibited the strongest APC peak suggesting a lack of cell replication. However, when cultured with the CAF cells, the APC peak had shifted to the left, indicating reduced brightness and cell replication (Figure 10). Nevertheless, when repeated in the 72-hour migration assay, no reduced APC expression was detected, neither in the conditions with CAF cells nor with CAF CM.

DISCUSSION

Flow Cytometry

The flow cytometry data revealed that the U87 tumor cells and GBM CAFs cannot be differentiated. There were differences in GBM tumor cell and GBM CAF expression of the surface markers CD45, CD73, and CD140b, as hypothesized. It was also hypothesized that the GBM CAFs could be able to be differentiated from the U87 cells by the expression of the intracellular marker GFAP and the extracellular markers, CD29 and CD49e; however, there were no differences in expression of those markers. One interesting marker that was expressed by the GBM CAFs was GFAP, typically associated with astrocytes, which GBM CAFs do not express. GFAP supports the structures of astrocytes and the blood-brain barrier (Jurga et al., 2021). Nevertheless, it was listed as positive on the commercial data sheet for these primary human GBM CAFs and the positive expression of GFAP was confirmed in the flow panel.

Based on the conflicting flow cytometry data in CAF surface marker expression, differentiation between the U87 and GBM CAF cell types could not be made when co-cultured. Although there is no literature directly citing differences in the expression of these specific markers between GBM tumor cells and GBM

CAFs, separate studies have identified CAFs using these surface markers. This suggests that CAFs could be identified by these markers, although this was not confirmed in the current study. Additional markers have been used to identify CAFs and CAF-like cells, specifically fibroblast associate protein (FAP) which could be assessed in the future. Although the difference in surface marker expression would have allowed for the ability to distinguish between cell types in a patient GBM sample, that was not possible due to the high similarity between the two cell types, that was not possible. Subsequently, the GBM CAFs and U87 tumor cells were dyed with a membrane dye to differentiate between the two cells during experiments to establish the role of GBM CAFs in GBM cell migration.

Transwell Migration

Following the 24-hour and 72-hour U87 migration, the assay demonstrated that the presence of the GBM CAFs did induce U87 cell migration, as hypothesized. Compared to the control conditions, there was an increase in U87 cell migration through the transwell insert. Further, similar amounts of the U87 cells migrated towards the GBM CAF cells and the GBM CAF CM. GBM CAF soluble factors, like IL-6, may induce U87 migration, yet further studies are necessary to confirm the CAF soluble factors (Kitzberger et al., 2023). Nevertheless, these values were not statistically significant and need further optimization to clarify the relationship between GBM CAFs in GBM cell migration.

After IL-12 was supplemented to the GBM CAFs, the number of U87 cells that migrated dropped considerably. This may be due to the antagonistic interactions between IL-12 and GBM CAF cells themselves, as hypothesized. However, CAF CM that was supplemented with IL-12 exhibited greater U87 cell migration. Although the mechanism for this induced migration is unclear, it is hypothesized that IL-12 may directly act on GBM CAFs to change the secretion of soluble factors and subsequent GBM migration.

Limitations & Future Directions

A limitation of the study is that there are other cells and factors in the TME that influence GBM tumor

migration, aside from just GBM CAFs. NK cells and macrophages are just a few examples of cells that can influence GBM tumor cell migration, and their roles cannot be excluded when exploring GBM tumor cell migration. Additionally, although flow cytometry is effective when a different cell type as the unstained control, the vast differences in size and characteristics between GBM tumor cells and GBM CAFs suggest it may be more effective to use an unstained GBM CAF sample for comparison. Had the unstained GBM control been included, changes in expression of the surface markers of the GBM CAF cells may have been more clearly noted. Further, running flow cytometry on the same surface markers for a CAF originally from another cancer type, like breast cancer, may have also been useful in distinguishing the differences between GBM CAF and carcinoma CAF surface markers. Lastly, due to the GBM CAFs being commercially purchased, they may not be the best representation of GBM CAFs taken directly from a patient's TME. However, there are challenges in culturing GBM CAFs, as they require more care and time.

For future study designs, rather than using the commercially available GBM CAF line, dissociating GBM CAFs from a patient tumor sample will result in GBM CAFs that are more representative of cells that are directly interacting with GBM tumor cells in the TME. The commercially available GBM CAF line may have caveats due to some of the surface markers identified (like GFAP) that would not otherwise be found in the GBM CAF cells. Additionally, there must be greater efforts to optimize the transwell migration assay protocols. The interest of this project was of U87 cell migration, which made the number of CAFs present in the transwell irrelevant. However, upon counting the cells present in the assay, no CAFs were counted. This may be due to an error in the harvesting of the cells. Following transwell migration, the U87 and CAF co-culture can be collected to further explore the CAF soluble factors that may influence U87 migration. An enzyme-linked immunosorbent assay (ELISA) or multiplex ELISA like a LegendPlex would be a good approach to gain a better understanding of the soluble factors influencing U87 migration. ELISAs can detect specific cytokines and other soluble factors that are released; therefore, it may be useful to understand what

soluble factors CAFs are releasing which influence GBM tumor cell migration. Further, due to specific interests in the role of GBM tumor cell migration and growth due to the presence of CAFs in the brain, as well as the prevention of immune cell proliferation due to immune cell exhaustion markers and a physical barrier, a mouse study could be conducted. Injecting varying amounts of CAFs into the brain alongside the CT2A tumor cell line can help determine the effects of CAFs on tumor progression and prognosis.

CONCLUSION

With the pervasive interest in immunotherapies to treat patients with GBM, it is important to consider the GBM tumor microenvironment to optimize immune cell infiltration. GBM CAFs have been reported to exhibit similar characteristics as CAFs found in carcinomas and further have been determined to create an immunosuppressive environment surrounding the tumor and encourage angiogenesis and ECM modification in the GBM TME. The present study showed that some markers differentiated GBM cells from GBM CAFs, while others did not. GBM CAFs promoted GBM migration, with IL-12 reducing migration when applied to GBM CAFs. However, GBM migration was increased with added IL-12 in CAF-conditioned media. Refining assays and using patient-derived CAFs could identify key soluble factors, highlighting importance of the TME in optimizing GBM immunotherapy. Further studies are necessary to optimize transwell migration assay conditions and to understand the specific soluble factors through which the GBM CAFs are promoting U87 cell migration.

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Meet Our Editing Team

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Sasha Bacot,
Editor-in-Chief



Sasha (Trinity '25) is from South Carolina and is a double major in Biology and Computer Science. She loves being a Vertices peer reviewer because it allows her to delve deeper into what she's most passionate about: scientific research! Outside of her work with Vertices, Sasha loves to figure skate, listen to music, and try out all the cool restaurants in Durham (especially for boba)!

Kaeden Hill,
Editor-in-Chief



Kaeden (Trinity '25) is a Vertices Senior Editor from Atlanta, Georgia, double majoring in biology with a concentration in molecular and cell biology, and evolutionary anthropology with a minor in chemistry. After graduating, he plans to pursue a Ph.D. and a career in research. He is specifically interested in DNA tumor viruses and how their “cellular hijacking” can drive cells towards cancer, and he is a member of the Luftig Lab, studying Epstein-Barr virus and the cancers that it causes. Outside of academics, he loves to hike, travel, ski, scuba dive, collect minerals, and make jewelry.

**Eliza Goldstein,
Senior Editor**



Eliza Goldstein is a second-year undergraduate student majoring in Psychology with a minor in Global Health. She is interested in clinical and social psychology, specifically the intersections and variance of psychopathology among differing backgrounds and experiences. After completing her undergraduate degree, she plans to pursue a Ph.D. in Clinical Psychology. At Duke, she is the Lab Manager for the Culture Lab and a Research Assistant for the Zucker Lab. Outside of research, she enjoys biking, reading, and spending time with her friends.

**Vishruth Hanumaihgari,
Senior Editor**



Vishruth (Trinity '27) is from Allentown, Pennsylvania and plans to major in neuroscience. He loves doing research in the lab, and he joined Vertices because he can read other people's work and help them publish the best possible version of their projects. Outside of Vertices, Vishruth also loves to play basketball and try new foods!

**Eric Lee,
Senior Editor**



Eric is a junior from Jacksonville, Florida studying premedicine and computer science at Duke. He is particularly interested in the intersections of computational techniques and biomedical psychiatric research. On campus he is passionate about working with research education and mental health organizations. In his free time, he enjoys ping-pong, board/card games, cooking, and music.

PEER REVIEW TEAM

Isaac Ayo' Oyegbade



Isaac (Fuqua '25) is from western Nigeria and majors in Strategy & Corporate Finance for his MBA degree. Prior, he obtained a Biochemistry B.Sc. in 2015 and became a Chartered Accountant in 2022. He has worked for 9 years in Banking, Consulting & Finance across the EMEA & the U.S. Outside of Vertices, he is on Duke's Racial Equity Advisory Council and is the Vice-President of External Affairs & Alumni Engagement for the Duke MBA Consulting Club and the Business in Africa Club. He imagines himself a pro-pickleball player and an amateur skateboarder.

Emerson Cortazar



Emerson (Pratt '28) is from Charlotte, North Carolina and is majoring in Electrical and Computer Engineering. In his free time, he enjoys baking, reading books, and watching movies.

Daliya Rizvi



Daliya (Trinity '27) is studying biochemistry and applied ethics on the premed track. She is interested in the intersection of novel cancer treatments and the bioethical issues posed by long-term end-of-life care. Outside of Vertices, she is passionate about music, research, reading, and running track.

Jinny Guo



Jinny (Trinity '28) is from Pennsylvania and is majoring in Biology and Computer Science with a certificate in Innovation and Entrepreneurship. Outside of Vertices, she studies tissue regeneration after severe radiation injury in the Lee Lab, dances with Synergy Dance Company, and plans large on-campus events as part of DUU's Special Event Committee. Besides research, she enjoys swimming, running, dancing, and watching shows and movies in her free time.

Gage Gruett



Gage (Trinity '28) is from Austin, Texas and is majoring in Neuroscience with a minor in Art History and Chemistry. Outside of Vertices, he studies eating disorders in the Duke Center for Eating Disorders (DCED) Lab, optogenetics in the Mooney lab, and spearheads events for the National Alliance on Mental Illness (NAMI) as their Executive Publicity Officer. In his free time, he enjoys thrifting at local stores, ranking cafes and movies, and volunteering to help describes and curate art exhibits for incarcerated artists through Durham's local Impartial non-profit!

Hannah Hortman



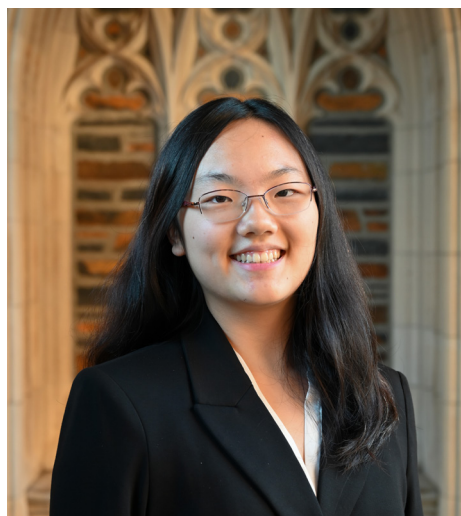
Hannah (Trinity '26) is from Raleigh, North Carolina and majoring in Neuroscience with minors in Computer Biology and Chinese. She studies therapeutic targets for Parkinson's Disease in the Calakos Lab. Beyond her work as a Vertices peer editor, Hannah enjoys dancing, figure skating, painting, and trying new restaurants!

Daniel Levin



Daniel is a sophomore from Pittsburgh, PA, studying chemistry and biology. His research at Duke focuses on understanding the role of noncoding RNA during immune responses. After graduating, he aspires to pursue a Ph.D. and research the chemical origins of life. Outside of the lab, Daniel enjoys ice skating and gardening.

Zishen Li



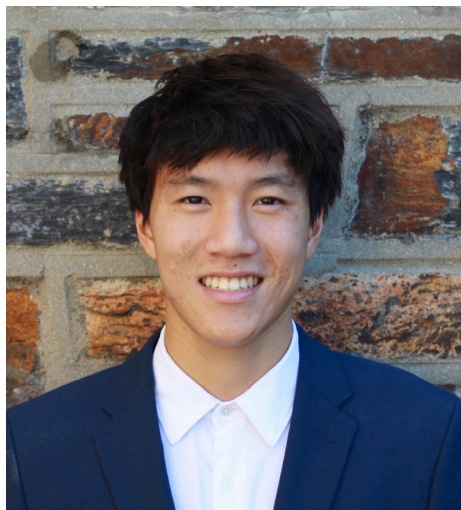
Zishen (Trinity '27) is from Xi'an, Shaanxi, China, planning on majoring in biology and minoring in visual arts on the pre-med track. Zishen is interested in medicine as well as how art and science intersect. Outside of Vertices, she is a part of the TIRTL Lab studying the immune responses to transplants. She also enjoys listening to music, acting with Duke Chinese Theater, playing ball sports, and exploring new things!

Krystal Liu



Krystal (Trinity '28) is from Nanjing, China, and plans to double major in History and Economics. She is very interested in History and many other humanities subjects. She loves being a Vertices peer reviewer because it allows her to explore her passion for research and discover the exciting work her peers are doing! Outside of Vertices, Krystal enjoys crafting, working out, and spending time with friends.

Max Niu



Max (Trinity '28) is from Marlboro, New Jersey and is majoring in computer science with a concentration in machine learning and marine science & conservation. He is specifically interested in the application of machine learning and other forms of technology in marine science. He is a part of the MaRRS Lab, conducting his own research project on leveraging machine learning to analyze data from whale tagging. Outside of academics, he loves playing pickleball and tennis, swimming, hiking, traveling, snowboarding, scuba diving, and weight lifting.

Anushka Peer



Anushka (Trinity '28) is majoring in Chemistry is from the Bay Area, California. She's specifically interested in microbiology and drug discovery and loves learning about new research fields through peer editing. Outside of Vertices, she enjoys volunteering in Hospice, playing the flute in the Duke Wind Symphony, and getting cookies from Insomnia!

Harry Pratt



Harry (Trinity '27) is from New York and is majoring in Biology with a minor in Chemistry on the pre-med track. Outside of Vertices, he studies neurodegeneration in the Sanders Lab and volunteers to address social determinants of health in a clinical setting with Help Desk. In his free time, he enjoys playing music, and is a drummer in a band on campus called The Trench.

Will Sun



Will (Trinity '27) is originally from San Jose, California, and is majoring in biology, with minors in neuroscience and marine science and conservation. He is interested in the intersections between marine biology and medicine, specifically marine immunology and pharmacology. In his free time, Will enjoys playing basketball, going on sunrise hikes, and collecting stickers.

Naomi Wordofa



Naomi (Trinity '28) is from Monterey, California and hopes to major in Biology and Global Health. Outside of school, she enjoys long-distance running, playing the violin and doing photography.

Rachel Wu



Rachel (Pratt '28) is a biomedical engineering and cs major from Omaha Nebraska. She is interested in computational biology and how machine learning can be used on biological data, and is currently working in the Hickey Lab. Outside of academics, she plays viola in the Duke Symphony Orchestra and enjoys exploring new restaurants with friends

Alice Zhang



Alice (Trinity '28) hails from Tianjin, China, and is majoring in Biology with a keen interest in receptor mechanisms and nanofiber immunotherapy delivery. Outside research, Alice is an astronomy enthusiast, is working toward her private pilot license, and enjoys applying aromatherapy!

Ura Zhang



A young but passionate biology researcher, public welfare initiator, outdoor enthusiasts, and scenery photographer.

Meet Our Design Team

**AJ Kochuba,
Artistic Director**



AJ (Trinity '25) is from Raleigh, North Carolina, studying neuroscience, psychology, and visual arts on the pre-med track. AJ is particularly interested in humanities-based approaches to medical practice and research, and they hope to enrich the synergy between science and the arts.

Gaby Dunn



Gaby is a junior from South Florida with too many interests that she is trying to find a major for. She is fascinated by the intersections of education, art, & STEM. When procrastinating, she enjoys rock climbing, critiquing movies, and spending time outdoors.

Amanda Li



Amanda (Pratt '27) is from Charlotte, North Carolina and is majoring in biomedical engineering. She loves learning about anything and everything, doodling in her spare time, and exploring new things.

Monet Shum



Monet (Trinity '27) is a sophomore from Aldie, Virginia planning to study linguistics, computer science, and classical civilizations. Outside of Vertices, she also works with Duke's Digital Art History and Visual Culture Lab and enjoys beading and walking in the Duke Gardens.

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