MAGEE-WOMENS RESEARCH INSTITUTE & FOUNDATION

THE MAGEE-WOMENS RESEARCH INSTITUTE CLINICAL TRAINEE RESEARCH AWARD

FACE PAGE (must be typewritten)

APPLICANT INFORMATION	
NAME (Last, first, middle) [redacted]	DEGREE(S): MD
	ARE YOU A RESIDENT OR FELLOW? Fellow
POSITION TITLE: [redacted]	OFFICE MAILING ADDRESS (building, room, street, city, state, zip code)
YEAR(S) IN TRAINING: [redacted]	[redacted]
YEAR(S) IN CURRENT PROGRAM: [redacted]	
DEPARTMENT Gynecologic Oncology	
TEL: [redacted] FAX: [redacted]	E-MAIL ADDRESS: [redacted]

APPLICATION TITLE: Targeting quiescent ovarian cancer cells with a novel antibody drug conjugate

HUMAN SUBJECTS RESEARCH	☑ No ☐ Yes	IRB APPROVAL DATE:
VERTEBRATE ANIMALS	☐ No ☑ Yes	IACUC APPROVAL DATE: 11/19/2020
TOTAL FUNDS REQUESTED	$5,000	

FACULTY SPONSOR	DEPARTMENT CHAIR OR DIRECTOR OF FELLOWSHIP/RESIDENCY PROGRAM
Name [redacted]	Name [redacted]
Title Professor of Medicine Director of Ovarian Cancer Center of Excellence	Title [redacted]
SIGNATURE [redacted]	SIGNATURE [redacted]

APPLICANT SIGNATURE	DATE
[redacted]	[redacted]

PROPOSED BUDGET

Personnel – List **effort** for **all** personnel to be involved in carrying out the proposed research, whether or not salary is requested, **beginning with P.I.**

Name	Role	% Effort	Salary Requested	Fringe Benefits*	Total
[redacted]					
	Subtotals		$	$	$

Equipment	SEC column	Subtotal	$280
Supplies (list)	Antibody-drug conjugate production: - PBD dimer with VC-PAB linker - anti-human CD24 antibody, IgG isotype control antibody - chemical reagents	Subtotal	$4720
Other Expenses		Subtotal	
		Total	$5,000

*MWRIF Staff fringe benefit rate is 25.0%

BUDGET JUSTIFICATION

The grant would support the funds necessary to develop and test a novel anti-CD24 antibody drug conjugate and test the efficacy in a murine model. In order to specifically target quiescent cells we will conjugate anti-CD24 to a PBD dimer, which is not a currently available commercial product and must be developed in the laboratory.

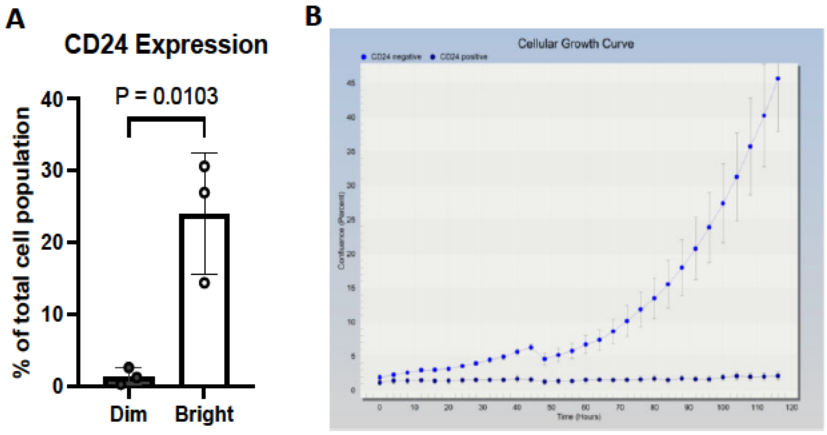
Abstract:
Despite advances in therapeutics, chemotherapy remains the mainstay treatment for ovarian cancer (OvCa). Unfortunately, most OvCa patients develop chemotherapy-resistant disease that is inevitably fatal. One understudied biologic mechanism of chemotherapy resistance is quiescence. Quiescent cells reversibly exit the cell cycle and are thus refractory to chemotherapies targeting rapidly proliferating cells. Indeed, studies in animal and human tumors suggest that quiescent cells can survive chemotherapy and re-enter the cell cycle, driving disease recurrence. One way to eradicate these cells is via antibody drug conjugates (ADC). The cell surface marker, CD24 is upregulated in quiescent ovarian cancer cells (qOvCaC). Furthermore, our preliminary data show that histone deacetylase inhibitors (HDACi) upregulate CD24 expression. We hypothesize a novel approach to induce CD24 expression with a HDACi coupled with an anti-CD24 ADC to eradicate CD24+ qOvCaC and increase cure rates. We propose to develop a novel anti-CD24 ADC and test this in combination with HDACi.

Training Goals:
After spending one year in the lab as part of her gynecological oncology fellowship, Dr. [redacted] has expressed an interested in developing a career as a translational physician scientist. The training goals of this grant are two-fold. First this project will allow Dr. [redacted] to continue to develop her basic science skills. This includes novel skills developing and testing an antibody drug conjugate as a therapeutic. Second, as this grant will allow Dr. [redacted] the opportunity to juggle clinical and lab work, while developing skills mentoring graduate students who will aid in aspects of the project.

Hypothesis: Induction of CD24 expression with an HDACi coupled with an anti-CD24 ADC can be used to eradicate CD24+ qOvCaC and increase cancer eradication rates.

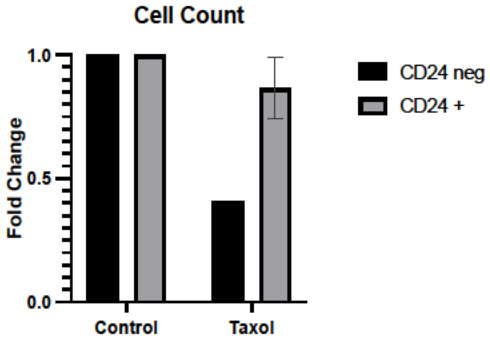
Significance: Ovarian cancer remains the deadliest gynecologic malignancy[1]. Despite recent advances in drug development, including PARP-inhibitors, around 70% of OvCa patients will recur[1,2]. Because most chemotherapeutic agents are aimed at targeting rapidly dividing cells, they fail to address a specific population of "persister cells" which are the qOvCaC. These quiescent cells can survive chemotherapy and re-enter the cell cycle and develop into clinically recognized tumors, driving recurrence. Ultimately, recurrent OvCa patients develop chemotherapy resistance which is fatal[2]. Consequently, new therapies to target these quiescent stem cells or prevent the growth of residual cancer cells post-chemotherapy are critically needed.

Figure 1: A) CD24 expression in cell trace violet dim versus bright cells. B) Cellular proliferation in CD24 negative vs CD24 positive cells.



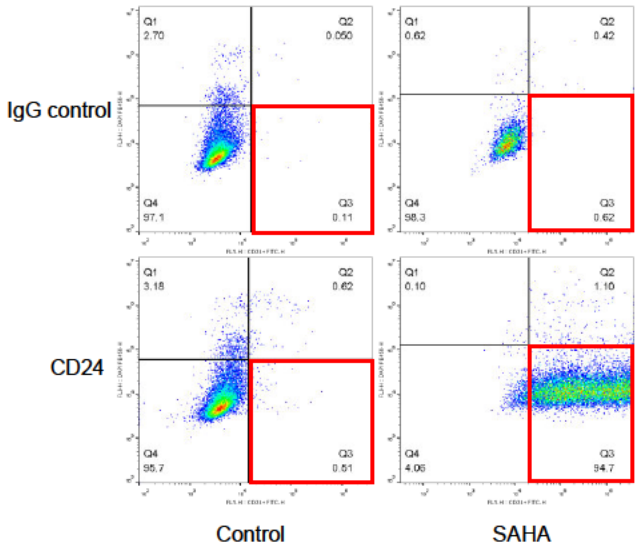
We suggest that (i) induction of quiescence in cancer cells that persists after chemotherapy can delay disease recurrence, and (ii) elimination of quiescent stem cells can increase tumor eradication to increase cure rates[3]. There is a growing body of evidence to suggest that CD24 is expressed on the cell surface of qOvCaC and thus a potential marker and therapeutic target for eradication of these cells[4,5]. Additionally, more recent published data suggests that CD24 is a signal to the immune system against phagocytosis, thereby escaping eradication by the immune system[6]. Our preliminary data confirm that CD24 is more highly expressed in a quiescent cell population. After staining with cell trace violet to track cellular division and proliferation, CD24 expression is higher in the cell trace violet bright population (has retained the dye and thus proves low/no cellular division) compared to the dim cells (multiple cell divisions to dilute the dye). Additionally, CD24 positive cells show much slower growth than CD24 negative cells in real-time quantitative live-cell imaging and analysis platforms [Figure 1]. Additionally, quiescence is also an important driver of chemotherapy resistance in OvCa, and we have shown that CD24+ cells are more resistant to chemotherapy[5] [Figure 2].

Figure 2: CaOv3 cell count after 48 treatment with paclitaxel



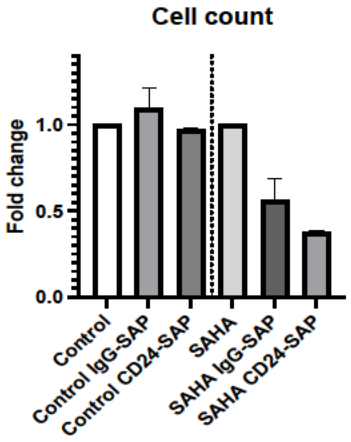
As CD24 is not expressed on all qOvCaC, an ADC targeting CD24 could be insufficient to eradicate all cells. To overcome this, we have identified mechanisms to induce CD24. We found that just 24 hours of treatment with the HDACi Vorinostat (SAHA), can induce expression of CD24 on >90% of OvCa cells [Figure 3]. Therefore, not only is CD24 innately expressed on qOvCaC, we can induce its expression with a quiescence inducing drug. Confirming an ADC can kill qOvCaC, we treated cells with Vorinostat (SAHA) and then treated with a biotinylated CD24 antibody coupled via streptavidin to the laboratory toxin saporin [Figure 4]. This preliminary data provides proof

Figure 3: CD24 expression with 48h treatment of Vorinostat.



of the principle, however saporin is not a clinically viable agent. While there are several clinically utilized drug conjugates, many (MMAE etc.) were developed to kill rapidly dividing cells and will not kill quiescent cells. However, pyrrolobenzodiazepine (PBD) dimers are DNA damaging and capable of killing non-dividing cells, including cancer stem cells, thus making them the ideal drug payload for quiescent cells[7].

Figure 4: Cell count normalized to group control after treatment with biotin antibody and streptavidin saporin.



Specific Aim 1: Develop a clinically applicable antibody drug conjugate (ADC) that targets quiescent cells.

1.1 Using an anti-cd24 antibody we will join it to a commercially available PBD dimer with valine-citrulline paminobenzylcarbamate (VC-PAB) cleavable linker. By using this product, we will be able to conjugate 5mg of antibody with a drug antibody ratio (DAR) of 3-4. The antibody will be dissolved in a buffer solution and added to the payload drug solution. After 3-hour reaction time the product will be filtered over a Millex membrane and purified using size exclusion chromatography. An IgG isotype control ADC will also be developed in a similar manner.

1.2 Evaluate the efficacy and validity of this ADC using in vitro cell culture of OvCa cell lines (PT340, OVSAHO, CaOV3) both pre-treated with SAHA and controls. The IC_{50} will be determined after analyzing cell counts mean +/- standard deviation of 5 different dilutions. Based on prior literature review we expect testing in vitro doses between 10-1000ng/ml. We will also perform flow cytometry for CD24 expression and apoptosis assays.

1.3 To measure the bystander effect, a cd24+ cell population will be treated with the anti-CD24 ADC and the conditioned medium will be collected after 48h. The medium will then be transferred to CD24- cells for incubation. Cell viability will be measured with cell counts and apoptosis assay.

Specific Aim 2: To evaluate the anti-tumor activity of the anti-CD24 ADC *in vivo.*

2.1 OvCa cells will be inoculated into the flank of immunocompromised mice and monitored until their growth reaches 50-100mm^3. Pre-treatment with either Vorinostat or traditional cytotoxic chemotherapy (carboplatin) will then take place. Mice will then be treated with anti-CD24 ADC vs murine IgG ADC control at a dose between 0.5-1mg/kg/dose given weekly. Tumor growth curves and metastasis will be evaluated. Euthanasia will take place when tumor volumes reach 1000mm^2 or other euthanasia criteria is met. Tumors will be snap frozen for immunohistochemistry. Frozen tumors will be stained for Ki67 to assess proliferation, CD24, and cleaved caspase 3 to assess apoptosis.

Impact:

At the conclusion of our studies, we expect to show that CD24 is a novel therapeutic target for OvCa, targeting qOvCaC that are chemoresistant with the goal of increasing cure rates and treatment options in recurrent disease. The next steps after successful *in vivo* murine models which can be used in support of initiation of a phase 1 clinical trial in OvCa.

Biostatistical Analysis:

Discrete variables such as cellular counts, percentage of cellular death and CD24 expression will be expressed as means +/- standard deviation and comparison between groups will be a two tailed t-test ($p<0.05$). For murine model tumor measurements, we have chosen n=10 animals per group based on final tumor volumes around 1000mm^3 with a standard deviation of 30%. This will allow for an 82.5% power to detect a 35% reduction in tumor volume growth, or a 95% power to detect a 50% reduction. Tumor growth between groups will be analyzed using repeated measures analysis of variance (ANOVA) techniques. All statistical analysis will be performed using GraphPad Prism 9 software.

Timeline:

This project is expected to take six months to complete and is therefore feasible for completion within the one year award period. I am currently in my second year of fellowship training and therefore will be able to complete the project prior to graduation from my fellowship program.

References:

1 Recurrence. Ovarian Cancer Research Alliance. https://ocrahope.org/patients/about-ovarian-cancer/recurrence/ (accessed Aug 21, 2022).

2 Cooke SL, Brenton JD. Evolution of platinum resistance in high-grade serous ovarian cancer. *Lancet Oncol* 2011; **12**: 1169–74.

3 Zhang J, Si J, Gan L, *et al.* Research progress on therapeutic targeting of quiescent cancer cells. *Artif Cells Nanomed Biotechnol* 2019; **47**: 2810–20.

4 Burgos-Ojeda D, Wu R, McLean K, *et al.* CD24+ Ovarian Cancer Cells Are Enriched for Cancer-Initiating Cells and Dependent on JAK2 Signaling for Growth and Metastasis. *Mol Cancer Ther* 2015; **14**: 1717–27.

5 Gao MQ, Choi YP, Kang S, Youn JH, Cho NH. CD24+ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells. *Oncogene* 2010; **29**: 2672–80.

6 Barkal AA, Brewer RE, Markovic M, *et al.* CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature* 2019; **572**: 392–6.

7 Ponziani S, Di Vittorio G, Pitari G, *et al.* Antibody-Drug Conjugates: The New Frontier of Chemotherapy. *Int J Mol Sci* 2020; **21**. DOI:10.3390/ijms21155510.

University of Pittsburgh
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Tel: 412-383-2008
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IACUC APPROVAL

Protocol #: 20118266
PHS Assurance Number: D16-00118

Principal Investigator:	Ronald Buckanovich
Protocol Title:	A humanized tumor model to study cancer biology and therapeutic development (2)
Additional Titles:	Developing Novel Cancer Therapies Studying ovarian cancer cell biology
Funding Source(s):	NIH (CA211913S1, CA218026, CA238315)
Approval Date:	11/19/2020

To Whom It May Concern:

The University of Pittsburgh's Institutional Animal Care and Use Committee has reviewed and approved the research proposal referenced above.

The committee finds that the protocol meets the standards for humane animal care and use as set by the Animal Welfare Act and the NIH Guide for the Care and Use of Laboratory Animals.

Sincerely,

Deborah L. Chapman, PhD
Institutional Animal Care and Use Committee

The three year term of this protocol will expire on 11/19/2023. A full de novo rewrite and review must be completed and approved before this date to continue the project after this protocol expires.

IS00018266