Tumor Glycolipids, a New Frontier for Early Detection and Precision Medicine in Ovarian Cancer and Other Malignancies

By Samantha S. Yee, Anna Milik Jeter and H. Uri Saragovi, PhD

Abstract

Ovarian cancer is the most lethal gynecologic cancer and fifth cause of cancer death in women in the U.S. due, in part, because there are currently no reliable ovarian cancer biomarkers that detect early stages of the disease. Therefore over 70% of patients are diagnosed at advanced stages when current treatments are too late to be effective. Despite the introduction of BRCA and HRD genotyping, cure rates have not improved meaningfully in the past four decades. In this paper, we report on the quantification of tumor glycolipids as a novel tool for early detection of ovarian and other cancers. We discuss how AKRIVIS GD™, a proprietary liquid biopsy assay for early detection of ovarian cancer, has demonstrated high sensitivity and specificity to address the urgent market need. In a final observation, the same tumor glycolipids exploited for diagnosis also offer interesting molecular targets for precision cancer immuno-therapy. Preclinical studies utilizing these targets have shown promising results.

Introduction and Motivation

Despite advances in available treatments, BRCA and HRD genotyping, surgical procedures, and post-operative care, ovarian cancer remains the most lethal gynecologic cancer and fifth overall cause of cancer death in women in the United States (U.S.).[1–3] In 2021 alone, 14,000 women will succumb to the disease with another 200,000 living with the diagnosis.[1,4] Lifetime ovarian cancer risk is 1 in 78 and lifetime risk of succumbing from the disease is 1 in 108.[4] Cure rates have not meaningfully improved in the past four decades

and long-term survival has remained low in spite of improved therapies.[5,6] Ovarian cancer is a priority of women's cancer due to extremely low survival rates (**Table 1**).[3] All of this is largely due to late diagnosis of the disease. The overall 5-year survival rate of ovarian cancer in advanced stages is a mere 17-39%; however, women diagnosed early (stages I-II) have a 5-year survival rate of 70-95% (**Table 2**).[2,3,7] The risk of ovarian cancer increases significantly around the age of 40, with median age at diagnosis being 63 (**Figure 1**).[7] It has been estimated that, if 75% of ovarian cancer cases could be detected in early stages of the disease, the number of women succumbing from this cancer would be reduced by half.[8] Today, over 70% of patients are diagnosed at advanced stages (III-IV) of disease due to a lack of an early diagnostic marker.[3] Ovarian cancer screening, whether in general or in high-risk populations, has not proven to be effective.[9–13] This urgent, unmet clinical need demands an affordable and improved surveillance method that enables earlier detection within the symptomatic population.[14]

Ovarian cancer screening has not been successful

One approach to early detection of ovarian cancer is to screen women in the general population before the onset of symptoms. Unfortunately, the largest study conducted to date, UK Collaborative Trial of Ovarian Cancer Screening,* failed to show a reduction in mortality.[11] UKCTOCS used a combination of Carbohydrate Antigen (CA-125) biomarker (Roche Holding AG, Basel, Switzerland) and transvaginal ultrasound (TVUS) annual screening.[11] In line with previous studies, these methods are simply inadequate to detect early-stage ovarian cancer due to their limited sensitivity and specificity.[2,11–13,15] As screening of the general population has not been successful, the alternative of screening high-risk groups (e.g., women with family history of ovarian cancer and *BRCA1* and *BRCA2* mutation carriers) was proposed. Unfortunately, little success has been achieved in these populations.[9,10]

High-risk population with *BRCA1* and *BRCA2* mutations urgently needs a more sensitive biomarker to detect ovarian cancer early

The highest risk of ovarian cancer occurs in women with germline mutations in *BRCA1* or *BRCA2* despite the fact that *BRCA1* and *BRCA2* mutations only contribute 5-15% of all ovarian cancer cases.[16] For women who bear one or the other of these mutations, the estimated lifetime risk of ovarian cancer is 39% to 46% in women with a *BRCA1* mutation, and 12% to 20% in women with a *BRCA2* mutation.[8,17] High-grade serous ovarian carcinoma (HGSOC) is the most common subtype in women with *BRCA1* or *BRCA2* mutations.[18,19] The proportion of women who underwent BRCA testing rose sharply over the last decade with an anticipated 25% year over year increase in the next five years.[20] Of the almost one million women with *BRCA1* or *BRCA2* mutation carriers in the U.S., only 14% of those who carry these mutations have been identified by genetic testing to date.[21,22]

The need for genetic testing and monitoring women will continue to grow. The standard of care for monitoring women with *BRCA1/2* mutations is an annual TVUS and CA-125 starting at 30 years old.[18,23–25] Once women have completed child bearing, typically around the ages of 35-40, the standard of care is prophylactic salpingo-oophorectomy, which removes the ovaries and fallopian tubes.[24,26]

While prophylactic salpingo-oophorectomy is effective, this surgical method of prevention is invasive, results in loss of fertility, induces early menopause, and requires hormone-replacement therapy. Clearly, this population urgently needs a more sensitive and specific biomarker assay to detect ovarian cancer early, avoid unnecessary prophylactic surgeries and initiate therapy earlier in the course of the disease.

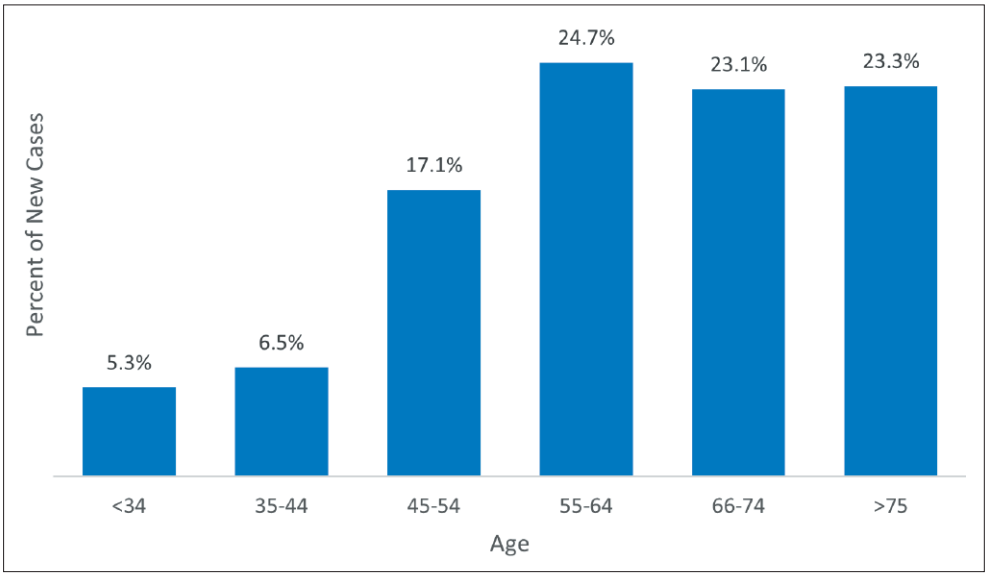


Figure 1: Percent of new ovarian cancer cases by age

Symptomatic patients are relegated to "wait and see" status

Ovarian cancer was labelled "the silent killer" due to the misconception that symptoms appear late in the disease process.[27,28] Results from twenty years of research has shown that women with ovarian cancer are symptomatic for many months before the diagnosis, including early-stage disease.[29–33] Over 90% of women reported experiencing multiple symptoms prior to their diagnosis.[2,3,32,34] Especially important, is that 87% of women with *early-stage* disease report experiencing symptoms, of which the vast majority (84.4%) consulted a health professional in the U.S.[2,3] Women who report symptoms to their provider such as bloating, pelvic pain, change in bowel movements, change in abdominal size, urinary frequency, back pain, and unexplained weight loss should be evaluated for ovarian cancer.[2]

Since early-stage symptoms are non-specific, they may be ignored, dismissed, or confounded with other conditions due to lack of an accurate biomarker test.[2,7] Lack of reliable tests leads to

Table 1: Ovarian cancer: a priority of women's cancer

Cancer Type	5-Year Survival
Breast	87%
Endometrial	79%
Cervical	67%
Ovarian	***46%***

Table 2: Five-year survival of Epithelial Ovarian Cancer in US by Stage

Stage at Diagnosis	5-Year Relative Survival
I	90%
II	70%
III	39%
IV	17%

a "wait and see" approach. Unfortunately, on average it takes 9 months to properly diagnose a symptomatic woman for ovarian cancer, with the U.S. having the longest delay-to-detection in the world.[3] Ovarian cancer grows quickly and can progress from early to advanced stages within a year.[35] With the most common form, malignant epithelial carcinoma, the cancer cells can grow out of control quickly leading to rapid proliferation of cancer cells that spread within weeks or months.[35] Indeed, more than 70% of patients are diagnosed at advanced stages of disease.[3] Consequently the 9-month delay translates to increased morbidity and mortality for most patients.

Better biomarker tests needed: A survey of available Assays

Blood-based Assays

As the statistical data cited in **Table 3** indicate, no ovarian cancer biomarker assay of sufficient specificity and sensitivity exists to detect early stages of the disease. For ovarian and fallopian tube cancers, early progenitor lesions are rarely identified because the location of the lesions makes direct visualization and sampling within the fallopian tubes difficult. This inability to visualize or obtain sample biopsy results in a "wait-and-see" approach, delaying diagnosis until an adnexal mass can be seen on imaging.[36–38]

Currently, CA-125 is the most commonly used biomarker; however, it is elevated in <50% of early-stage or unilateral ovarian cancers and is a more sensitive indicator of disseminated disease in ovarian cancer patients.[39,40] CA-125 is FDA cleared specifically to aid in detection of residual or recurrent ovarian cancer after treatment; this marker is also used to monitor disease progression.[41] However, despite the limited regulatory clearance, it is often used as an aid in ovarian cancer diagnosis.[2]

HE4 (Roche Holding AG, Basel, Switzerland) is a marker that has been investigated for superiority to CA-125 for both early detection and surveillance of ovarian cancer. Similar to CA-125, the HE4 marker is also only FDA cleared as an aid in detection of residual or recurrent ovarian cancer after treatment and for monitoring disease.[42] Some limitations of HE4 are similar to CA-125, as it is non-tumor or organ-specific and results are frequently confounded by false positives due to factors such as age; elevation in non-ovarian and non-malignant cases (e.g. renal failure); and liver cirrhosis which can cause a false positive result.[40,43,44]

The Risk of Ovarian Malignancy Algorithm (ROMA, Roche Holding AG, Basel, Switzerland) was introduced to improve differentiation of benign from malignant adnexal masses scheduled for surgery with a combination of HE4 and CA-125 data with menopausal status.[40,45] While, the sensitivity and specificity for ROMA are an improvement from a CA-125 result alone, it is still not sufficiently accurate to provide women with confidence for a sensitive and specific diagnostic for early detection of ovarian cancer, as an adnexal mass must be first be detected.

The OVA1 algorithm (Aspira Women's Health Inc, Austin, TX) uses a combination of five biomarkers: apolipoprotein A-1 (Apo A-1), transthyretin, beta-2-microglobulin, transferrin (TRF) and CA-125. Similar to ROMA, OVA1 is a risk malignancy index for adnexal masses scheduled for surgery.[46] It improves sensitivity over standard clinical assessment, but at a significant drop of specificity, which may lead to an increase in unnecessary procedures.[47–49] And, of course, the adnexal mass must first be detected, creating a rate-limiting step in the process.

"Especially important, is that 87% of women with early-stage disease report experiencing symptoms, of which the vast majority (84.4%) consulted a health professional in the U.S. Women who report symptoms to their provider such as bloating, pelvic pain, change in bowel movements, change in abdominal size, urinary frequency, back pain, and unexplained weight loss should be evaluated for ovarian cancer."

Ultrasonography

Transvaginal Ultrasound (TVUS) is often used for identification of complex adnexal masses for detection of ovarian cancer. TVUS focuses on the uterus which includes the fallopian tubes and ovaries allowing clinicians to visualize an abnormal shape or mass; however, it is not always clear whether a mass is malignant or benign.[40] HGSOC typically generated from lesions, that are not visible on imaging, shed into the peritoneal cavity allowing for advanced disease and metastasis to occur. HGSOC that present with peritoneal findings and regions other than around the uterus should be taken in consideration.[40,50,51] Evidence from modeling suggests that ovarian tumors need to be found when they are relatively small, considerably smaller than the current threshold used for transvaginal ultrasound (10 cm^3 for cysts) to be in an early stage at detection.[13,52] Thus, the effectiveness of ultrasonography is limited for early detection of ovarian cancer.[53]

Table 3: Existing approved methods for ovarian cancer detection

Methods	Sensitivity (%)	Specificity (%)	Description
Ultrasound (IOTA Simple Rules)[54]	88	91	Regression analysis of sonographic features Designed to allow preoperative assessment of ovarian masses by nonexpert sonographers by creating standardized definitions and terminologies that describe morphological features of any ovarian mass
Ultrasound (Pattern Recognition)[54]	88	93	Performed by expert sonographers who subjectively assess an ovarian mass to predict its underlying pathology
CA-125[57]	80	82	23 to 50% of stage I cases sensitivity[58] Elevated serum CA-125 levels may be observed in other physiological or pathological conditions (menstruation, pregnancy, endometriosis, inflammatory diseases of the peritoneum)[59]
HE4[57]	75	83	Levels vary in smokers and in hormonal contraceptive users Sensitivity declines in post-menopausal patients
ROMA algorithm[57]	85	80	Algorithm of CA-125, HE4 and clinical factors Cannot be used for standalone diagnosis Ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist
OVA1[60]	93	43	Algorithm of CA-125, transferrin, transthyretin (prealbumin), apolipoprotein A-1, and beta-2 microglobulin plus clinical factors Cannot be used for standalone diagnosis Ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist

IOTA: International Ovarian Tumor Analysis; ROMA: Risk of Ovarian Malignancy Algorithm

A recurring drawback of the existing ultrasonography methods is the relative lack of sensitivity and specificity. The lack of sensitivity does not allow detecting ovarian cancer at an early stage, leading to poor prognosis and survival for ovarian cancer patients. Just as bad, the lack of specificity may lead to unnecessary surgeries. Although, ultrasound algorithms such as International Ovarian Tumor Analysis (IOTA) and Pattern Recognition report high specificity, 60% of surgeries undertaken by Gynecology Oncologists for suspicion of ovarian cancer are benign masses.[54,55] The current standard of care methodologies lead to unnecessary surgeries (and anxieties) which have an economic impact on the healthcare system. Unnecessary surgeries may bring complications and significant costs, with procedure costs up to $16,000.[13,56] They also have a large emotional burden on the patient. So earlier detection and precise diagnosis will not only be lifesaving, but also cost saving.

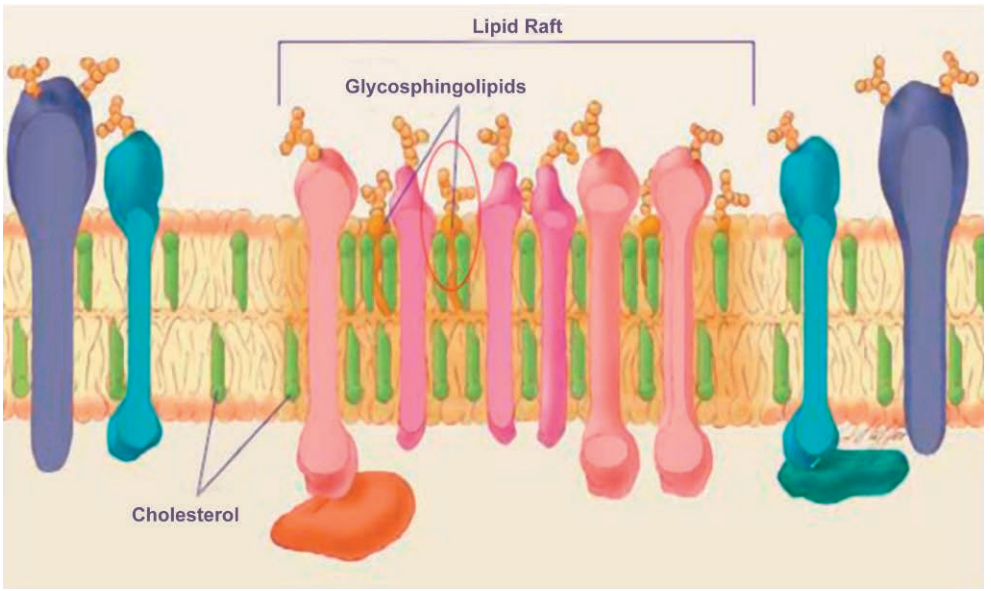


Figure 2: Simplified visual of a cell membrane with glycoproteins and glycolipids
Cell membrane depicting a lipid bilayer with (glyco)proteins and (glyco)lipids incoporated within (adapted from http://publications.nigms.nih.gov/insidethecell/chapter2.html).

Pan-cancer screening as an option

Pan-cancer screening has gained some interest recently due to assays such as CancerSEEK (Exact Sciences, Madison, WI), which tests for cancers across organs from a single blood draw and is intended to be undertaken alongside regular cancer screening. Looking specifically at ovarian cancer, CancerSEEK used existing cancer biomarkers such as CA-125 at much higher thresholds (>16x as high as that commonly considered the upper limit of normal) in addition to reflexing to a supporting PET Scan.[61] Over 50% of PET Scans conducted for those with a positive blood signal did not find disease present.[61] All existing biomarkers used in this test are known for low specificity and are not recommended for screening due to increased follow up, imaging, biopsy and potential surgery.[61] Overall, the results from CancerSEEK translated into much lower sensitivity in prospective data.[61] The addition of the PET Scan has led to re-evaluation due to its current cost of $8,000-10,000 and will likely be prohibitive as reflex testing.

Galleri (Illumina Inc., San Diego, CA) uses targeted methylation analysis of circulating cell-free DNA (cfDNA) to detect and localize >50 cancer types.[62] Galleri is also intended to be used in conjunction with other cancer screening tests.[63] The interim results reported sensitivity across 12 cancers at 34% at stage I and up to 77% at stage II, although no metrics are provided for ovarian cancer by stage. The overall sensitivity reported for ovarian cancer is 67% (CI 47-83%) with a specificity of 99%.[64] Despite increasing investigation on DNA methylation biomarkers for ovarian cancer, novel and large-scale studies will still need to be conducted to see if population screening will make an impact on overall mortality rates.

Tumor glycolipids, a new frontier of precision medicine in ovarian cancer and other malignancies

In order to address this urgent market need, AOA Dx Inc (Boston, MA) is developing a proprietary liquid biopsy assay, AKRIVIS GD™ for early detection of ovarian cancer. AKRIVIS GD is research use only undergoing further clinical trials and will be pursuing regulatory authorization. The assay involves a novel method of quantification of tumor glycolipids from a blood sample. Given their persistent and homogeneous expression almost exclusively in cancer, tumor-associated glycans are promising targets to be exploited as biomarkers. To date, genomics and proteomics have been used as tools in precision medicine. We foresee glycomics of tumor marker gangliosides as the next frontier used for diagnosis, prognosis, monitoring and eventually targeted therapies.

All cells are covered with a dense coat of glycans which are chains of carbohydrates bound to proteins or lipids.[65–67] Glycolipids are lipids with sugar residues of variable length and structure covalently attached and are a component of the lipid membrane (**Figure 2**). Glycolipids are found on the surface of all eukaryotic cell membranes.[65–68] An essential role of glycolipids is to maintain cell membrane stability and facilitate cellular recognition. Glycolipids are crucial to the immune response and in the cell-to-cell communication as connection for tissue formation.[66,69,70] To immune cells, surface glycans serve as an identifying feature of a cell and whether the cell should be removed from the human body if it appears harmful or foreign.[70]

Gangliosides are a family of >40 different sialic acid-containing glycosphingolipids. Each glycan tree is structurally unique and defines each ganglioside by name. Some gangliosides are ubiquitous and are present in normal cells, whereas other gangliosides are tumor markers, which are low/absent in normal cells and expressed at high levels in embryonic tissue and in cancer.[71–77] Changes in the expression of certain species of gangliosides have been described to occur during cell proliferation, differentiation, and ontogenesis.[72]

Aberrant and elevated expression of gangliosides has been also observed in different types of cancer cells. A subset of gangliosides, referred to as tumor marker gangliosides (TMGs), comprises a family of about 20 different gangliosides that are present preferentially or almost exclusively and at high density on the cell surface of certain cancers.[66] As the term "tumor marker" suggests, TMG expression is tightly associated with malignant cells. Expression of tumor gangliosides provides a survival advantage to the tumor, as some TMGs are known to afford tumors with immune evasion or immunosuppression, growth advantages, and better blood supply, all of which promote tumor growth, metastasis, and survival.[72] Moreover, gangliosides are actively released from the membrane of tumor cells, having a strong impact on impairing anti-tumor immunity.[72]

The active role of TMGs during cancer development makes them specific and etiological

biomarkers of malignancy.[66] Furthermore, TMGs are present on the tumor cell surface and can be shed into the extracellular environment.[78–86] TMGs are high value etiological biomarkers in tissue and serum, but until recently, they have been underexploited for diagnosis of any cancer.

"The active role of Tumor Marker Gangliosides (TMGs) during cancer development makes them specific and etiological biomarkers of malignancy.[66] Furthermore, TMGs are present on the tumor cell surface and can be shed into the extracellular environment."

Next Steps and Summary

AOA has developed a novel way to quantify TMGs in blood. The early data shows that this proprietary technology is able to detect cancers at early stages when they are still localized. Early detection of cancer can lead to better prognosis and survival, cost savings through reductions in unnecessary surgery, and potentially lower recurrence rates. AOA's first focus is on the development of AKRIVIS GD, a proprietary liquid biopsy assay for early detection of ovarian cancer. In an early proof of concept study of 72 patients, AKRIVIS GD showed sensitivity of 98% for detection of ovarian cancer and 93% for detection of early-stage disease.[87] A larger study consisting of 450 biobanked patient samples is ongoing with full results expected to be published in 2022.

Our results indicate that TMGs have high potential to be used in precision medicine approaches for guiding therapies and developing novel treatments. AKRIVIS GD will be positioned to fulfill the urgent and unmet need for surveillance of women with early signs and symptoms of ovarian cancer. We expect TMG assays will have further potential for minimal residual disease monitoring and population screening applications.

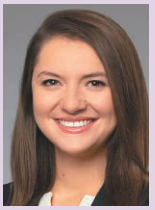
Providing clinicians with effective and affordable tools for early detection of ovarian cancer will go a long way in eradicating the stigma that ovarian cancer is a "silent killer". Centers for Medicare & Medicaid Services (CMS) already reimburses OVA1 and ROMA algorithms, therefore similar coverage should follow for AKRIVIS GD based on superior performance and earlier detection of ovarian cancer which may lead to better outcomes. Enabling affordable early-stage ovarian cancer detection could ensure equitable treatments in women's healthcare and be life-changing for many women. We will expand on this approach and discuss implications in a separate paper. IoPM

Summary Points

- Ovarian cancer is the most lethal gynecologic cancer and fifth cause of cancer death in women in the U.S.
- There are currently no reliable ovarian cancer biomarkers that detect early stages of the disease.
- AKRIVIS GD™, a proprietary liquid biopsy assay for early detection of ovarian cancer, has demonstrated high sensitivity and specificity to address the urgent market need.
- Quantification of tumor marker gangliosides may be a novel biomarker for early detection ovarian cancer and other cancers.

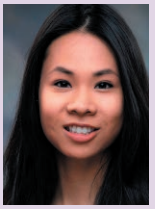
H. Uri Saragovi, PhD, Chief Scientific Officer, AOA Dx Inc.

Prof. Saragovi has been recognized as a world-leading scientist in the field of Translational Medicine. He is a leader in the field of Glycomics. He has published over 200 highly referenced scientific publications and 24 patents. He has also successfully translated drugs that were developed fully in his academic laboratory at McGill University, Lady Davis Institute Jewish General Hospital, in Montreal, QC, Canada. Research in his laboratory focuses on understanding macromolecular structure function relationships and receptor-ligand interactions with the aim of developing pharmacologically active peptidomimetics. Dr. Saragovi obtained his PhD from the University of Miami, FL in Molecular Immunology, Cell Biology, and Molecular Biology.



Anna Milik Jeter, Chief Business Officer & Co-Founder, AOA Dx Inc.

Anna is a dynamic business leader with a passion for advancing healthcare through world-class diagnostics fulfilling AOA's scientific mission and product development. Her strategic leadership provides scientific direction on biomarker strategies, clinical feasibility, and regulatory requirements for early cancer detection.



Samantha S. Yee, PhD Candidate, University of Texas Health San Antonio

Samantha is a fifth year PhD student in Dr. April L. Risinger's Lab in the Department of Pharmacology at UT Health San Antonio, and an NCI F99 fellow. Her dissertation research focuses on the use of microtubule targeting agents in women's cancers, particularly breast and ovarian. Sam is highly motivated to better understand the mechanisms of drug resistance in ovarian cancer to identify precision medicine therapeutics for patients.

Footnote/References

* UKCTOCS: 202,638 women enrolled and randomly assigned for screening or no screening groups

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