The Rivkin Center for Ovarian Cancer &
the American Association for Cancer Research present the 12TH Biennial

OVARIAN CANCER RESEARCH SYMPOSIUM

September 13–15, 2018 • Seattle, Washington

KANE HALL • UNIVERSITY OF WASHINGTON

INDEX

PROGRAM AND PRESENTERS' ABSTRACTS

Rivkin Center for Ovarian Cancer

AACR American Association for Cancer Research FINDING CURES TOGETHER

WELCOME

This year, the Rivkin Center for Ovarian Can¬cer is proud to partner with the American Association for Cancer Research (AACR) to present the 12th Biennial Ovarian Cancer Research Symposium. Both organizations have made significant investments in ovarian cancer research and strongly support this community of clinicians and researchers in sharing their ideas and collaborating so that meaningful breakthroughs can be achieved more quickly. In fact, AACR's support of the Scholar-in-Training Awards has made it possible for young investigators to join the conversation at the Symposium.

We thank the co-chairs—Drs. Frances R. Balkwill, Mary L. (Nora) Disis, Pamela S. Ohashi, and Elizabeth M. Swisher—for their hard work and guidance on assembling the exciting program ahead of us. It is an honor to gather distinguished speakers from around the world to share their knowledge on the basic science, prevention, early detection, and treatment of ovarian cancer.

We are pleased to again incorporate Scholars from the Ovarian Cancer Academy, funded by the Department of Defense, into the Symposium Planning Committee this year. The Rivkin Center, AACR, and the Ovarian Cancer Academy are all dedicated to funding and nurturing the next generation of ovarian cancer research leaders. The Scholars will also be hosting *Meet the Experts* roundtable discussions and will summarize the meeting for publication in collaboration with Dr. Deborah Armstrong, member of the Rivkin Center Scientific Advisory Board. We hope you will have a chance to meet a few of the Scholars during the Symposium.

We welcome and thank the patients, survivors, and advocates who are here at the Symposium for helping us to remember the reasons we are all here in the fight against ovarian cancer.

Now in its 24th year, the Symposium is the longest-running scientific meeting in the U.S. focused exclusively on ovarian cancer. We are grateful to be on the University of Washington's beautiful campus so that we may accommodate the growing number of attendees coming from all over the U.S. and the world.

We hope that you take away with you novel ideas to inspire your work, new friends with whom you can collaborate, and pleasant memories of Seattle.

Sincerely,

Joe White, MBA
Executive Director
Rivkin Center for Ovarian Cancer

Margaret Foti, PhD, MD (h.c.)
Chief Executive Officer
American Association for Cancer Research

SYMPOSIUM EVALUATION
Please go to ***https://www.rivkin.org/symposium-evaluation*** to complete the symposium evaluation after the close of the symposium.

PLANNING COMMITTEE

Frances Balkwill, PhD
(Co-Chair)
Barts Cancer Institute (United Kingdom)

Mary L. (Nora) Disis, MD
(Co-Chair)
University of Washington

Pamela Ohashi, PhD
(Co-Chair)
Princess Margaret Cancer Center (Canada)

Elizabeth Swisher, MD
(Co-Chair)
University of Washington

Amy Baran, PhD
American Association for Cancer Research

Kiran Dhillon, PhD
Rivkin Center for Ovarian Cancer

Yang Yang Hartwich, PhD
Yale University

Neil Johnson, PhD
Fox Chase Cancer Center

Karen McLean, MD/PhD
University of Michigan

Geeta Mehta, PhD
University of Michigan

Anirban Mitra, PhD
Indiana University School of Medicine

Saul Rivkin, MD
Rivkin Center for Ovarian Cancer

Juan Ruiz, PhD
Weill Cornell Medical College

Wa Xian, PhD
University of Texas, Houston

Dmitriy Zamarin, MD/PhD
Memorial Sloan Kettering Cancer Center

KEYNOTE SPEAKER

Mary-Claire King, PhD
University of Washington

INVITED SPEAKERS

Frances Balkwill, PhD
Barts Cancer Institute (United Kingdom)

Kathleen Cho, MD
University of Michigan

Alan D'Andrea, MD
Dana Farber Cancer Institute

Jung-Min Lee, MD
National Cancer Institute

Ernst Lengyel, MD/PhD
University of Chicago

Douglas Levine, MD
New York University Langone

Ranjit Manchanda, PhD
Barts Cancer Institute (United Kingdom)

Ursula Matulonis, MD
Dana Farber Cancer Institute

Brad Nelson, PhD
British Columbia Cancer Agency (Canada)

Rosana Risques, PhD
University of Washington

Elizabeth Swisher, MD
University of Washington

HELP SPREAD THE WORD:

Please join us in spreading the word about the 12th Biennial Ovarian Cancer Research Symposium on social media by sharing what you've learned, who you've met, and why you're excited about the future of ovarian cancer research. Use hashtag **#OvCa2018** so others can easily follow along. Every social media post helps raise awareness about ovarian cancer and the extraordinary work being done in ovarian cancer research.

We'll be sharing updates along the way as well. Follow, share, or tag the profiles below:

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#OvCa2018

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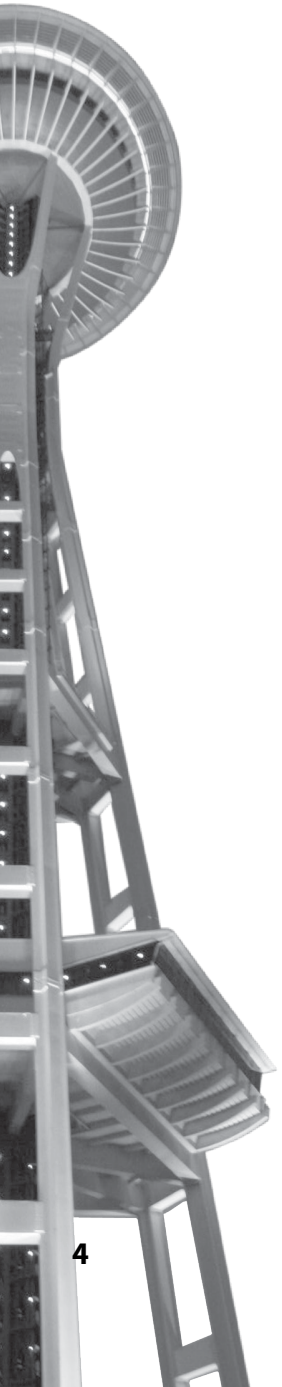


OVARIAN CANCER: PERSPECTIVES AND CURRENT CHALLENGES – KANE HALL

Biology of Ovarian Cancer — 4:15 PM
Douglas Levine, MD • *New York University Langone*

Tumor Microenvironment and Immunology of Ovarian Cancer — 4:40 PM
Brad Nelson, PhD • *British Columbia Cancer Agency (Canada)*

Clinical Management of Ovarian Cancer — 5:05 PM
Elizabeth Swisher, MD • *University of Washington*

KEYNOTE PRESENTATION — 5:30 PM

TBA
Mary-Claire King, PhD • *University of Washington*

POSTER SESSION I & RECEPTION HUB Ballroom (Husky Union Building) — 6:30–8:15 PM

Except where otherwise noted, all oral presentation sessions will be in Kane Hall.
Please see the walking route on the back cover.

SESSION 1 — DETECTION & PREVENTION OF OVARIAN CANCER – KANE HALL

7:00 AM — **REGISTRATION AND BREAKFAST** HUB Ballroom (Husky Union Building)

8:15 AM — **WELCOME** Kane Hall

8:30 AM — INVITED SPEAKER PRESENTATION

POPULATION TESTING FOR OVARIAN CANCER GENE MUTATIONS FOR PRIMARY PREVENTION
Ranjit Manchanda, MD/PhD • *Barts Cancer Institute (United Kingdom)*

9:10 AM — INVITED SPEAKER PRESENTATION

OVARIAN CANCER DETECTION USING ULTRA-SENSITIVE SEQUENCING: CHALLENGES AND OPPORTUNITIES
Rosana Risques, PhD • *University of Washington*

ABSTRACT SESSION

9:50 AM

LINEAGE CONTINUITY BETWEEN EARLY TUBAL SEROUS PROLIFERATIONS (ESPS/STILS) AND DISSEMINATED HIGH-GRADE SEROUS CARCINOMAS: A MODEL FOR "PRECURSOR ESCAPE"
Thing Rinda Soong, MD,PhD, MPH • *University of Washington*

10:05 AM

EXPOSING THE LOCAL FACTORS THAT CONTRIBUTE TO OVARIAN COLONIZATION OF FALLOPIAN TUBE-DERIVED TUMORS
Joanna Burdette, PhD • *University of Illinois-Chicago*

10:20 AM — **BREAK**

ABSTRACT SESSION CONTINUED

10:40 AM

DISTINCT CELL/TISSUE SOURCES OF HIGH-GRADE SEROUS OVARIAN CANCER
Shuang Zhang, PhD • *NYU School of Medicine*

10:55 AM

INCREASING ACCESS TO GENETIC TESTING FOR HEREDITARY BREAST AND OVARIAN CANCER: EXPERIENCE FROM HIGH-VOLUME OB/GYN CLINIC IN TEXAS
Carmelina Heydrich, MS • *Color Genomics*

11:10 AM

OVARIAN CANCER-ASSOCIATED RAD51D MUTATIONS WHICH IMPAIR ITS INTERACTION WITH XRCC2 RESULT IN DNA REPAIR DEFICIENCY
Kara Bernstein, PhD • *University of Pittsburgh School of Medicine*

11:25 AM

METABOLIC SYNDROME AND RISK OF OVARIAN CANCER IN THE USA: AN ANALYSIS OF LINKED SEER–MEDICARE DATA
Kara Michels, PhD • *National Cancer Institute, NIH*

11:40 AM

PROTEIN AND IMAGING MARKERS FOR EARLY DETECTION OF EPITHELIAL OVARIAN CANCER
Jennifer Barton, PhD • *University of Arizona*

11:55 AM

DEVELOPMENT OF A MULTI-PROTEIN CLASSIFIER FOR OVARIAN CANCER DETECTION BY SIMULTANEOUS MEASUREMENT OF 92 SERUM PROTEINS ON PROSEEK MULTIPLEX ONCOLOGY II PLATES
Amy Skubitz, PhD • *University of Minnesota*

12:10 PM –1:35PM — **LUNCH BREAK** HUB Ballroom (Husky Union Building)

12:30 PM –1:20PM — **Meet the Experts Roundtable Discussions**

Except where otherwise noted, all oral presentation sessions will be in Kane Hall.

SESSION 2 — GENOMICS AND MOLECULAR MECHANISMS OF OVARIAN CANCER – KANE HALL

1:35 PM — INVITED SPEAKER PRESENTATION

DNA REPAIR IN OVARIAN CANCER
Alan D'Andrea, MD • *Dana Farber Cancer Institute, Harvard Medical School*

2:15 PM — INVITED SPEAKER PRESENTATION

MODELING THE GENOMICS OF HIGH-GRADE SEROUS CARCINOMA IN THE MOUSE
Kathleen Cho, MD • *University of Michigan*

ABSTRACT SESSION

2:55 PM

COPY-NUMBER SIGNATURES AND MUTATIONAL PROCESSES IN HIGH GRADE SEROUS OVARIAN CARCINOMA
James Brenton, MD, PhD • *University of Cambridge (United Kingdom)*

3:10 PM

REAL-TIME ASSESSMENT OF HGSC DNA DAMAGE REPAIR DEFECTS AND DEFECT-INDUCED RESPONSE TO THERAPY IN OVARIAN CANCER ORGANOIDS
Sarah Hill, PhD • *Dana Farber Cancer Institute*

3:25 PM

IDENTIFYING FUNCTIONAL NONCODING SOMATIC MUTATIONS IN OVARIAN CANCER
Rosario Corona, MD, PhD • *Cedars-Sinai Medical Center*

3:40 PM — **BREAK**

ABSTRACT SESSION CONTINUED

4:00 PM

FOXM1 INDUCES DNA REPLICATION STRESS AND PROMOTES GENOMIC INSTABILITY DOWNSTREAM OF CYCLIN E1 IN HIGH-GRADE SEROUS OVARIAN CANCER
Carter Barger, PhD • *University of Nebraska Medical Center*

4:15 PM

GENOME-SCALE CRISPR KNOCKOUT SCREEN IDENTIFIES TIGAR AS A MODIFIER OF PARP INHIBITOR SENSITIVITY
Jeremy Chien, PhD • *University of New Mexico*

4:30 PM

POOLED GENOMIC SCREENS IDENTIFY ANTI-APOPTOTIC GENES AS MEDIATORS OF CHEMOTHERAPY RESISTANCE IN OVARIAN CANCER
Elizabeth Stover, PhD • *Dana-Farber Cancer Institute*

4:45 PM

IDENTIFICATION OF P53 PRIONS AS AN INDEPENDENT PROGNOSTIC MARKER FOR SURVIVAL IN HIGH-GRADE SEROUS OVARIAN CANCER
Nicole Heinzl, PhD • *Medical University of Vienna (Austria)*

5:00 PM

MODELING ENDOMETRIOID AND HIGH GRADE SEROUS CARCINOMAS IN THE MOUSE USING CRISPR/CAS9-MEDIATED SOMATIC GENE EDITING IN FALLOPIAN TUBE EPITHELIUM
Rong Wu, PhD • *University of Michigan Medical School*

5:15–6:30 PM

PANEL: ROLE OF ADVOCATES IN OVARIAN CANCER RESEARCH
Jamie Crase | Nita Maihle, PhD • *Augusta University* | Marcie Paul | Celeste Pearce, PhD, MPH • *University of Michigan*

6:30–8:15 PM — **POSTER SESSION II & RECEPTION** HUB Ballroom (Husky Union Building)

Please see the walking route on the back cover.

SESSION 3 — TUMOR MICROENVIRONMENT AND IMMUNOLOGY OF OVARIAN CANCER – KANE HALL

7:00 AM — **Registration and Breakfast** HUB Ballroom (Husky Union Building)

8:15 AM — **Welcome** Kane Hall

8:30 AM — INVITED SPEAKER PRESENTATION

Deconstructing and Reconstructing the Microenvironment of High-Grade Serous Ovarian Cancer
Frances Balkwill, PhD • *Barts Cancer Institute (United Kingdom)*

9:10 AM — INVITED SPEAKER PRESENTATION

Multi-Level Proteomics Identifies Cancer Testes Antigens as Mediator of Chemo Sensitivity and Immunotherapy Target in Ovarian Cancer
Ernst Lengyel, MD, PhD • *University of Chicago*

ABSTRACT SESSION

9:50 AM

Molecular and Functional Heterogeneity of Cancer Associated Fibroblasts in High-Grade Serous Ovarian Cancer
Laurie Ailles, PhD • *University Health Network (Canada)*

10:05 AM

Pro-Invasive Tumour-Stroma Interactions: Role of the Secreted Oxidoreductase CLIC3
Sara Zanivan, PhD • *CRUK Beatson Institute (United Kingdom)*

10:20 AM — **BREAK**

ABSTRACT SESSION CONTINUED

10:40 AM

Collagen Alterations in Human Ovarian Cancer Probed by Second Harmonic Generation (SHG) Imaging Microscopy
Paul Campagnola, PhD • *University of Wisconsin-Madison*

10:55 AM

Stromal Cell Expression of the Receptor Tyrosine Kinase DDR2 Promotes Ovarian Cancer Metastasis
Katherine Fuh, MD, PhD • *Washington University*

11:10 AM

A Novel Target for Overcoming Adaptive Resistance to Anti-Angiogenic Therapy in Ovarian Cancer
Yunfei Wen, PhD • *The University of Texas, MD Anderson Cancer Center*

11:25 AM

DNA Damage Responses and Immune Profiling Through Highly Multiplexed Tissue Immunofluorescence (t-CycIF) in High-Grade Serous Ovarian Cancer
Anniina Farkkila, MD, PhD • *Dana Farber Cancer Institute*

11:40 AM

Early Loss of Monoubiquitylation of Histone H2B Alters Key Immune Signaling Pathways Promoting the Progression of High-Grade Serous Ovarian Cancer
Jagmohan Hooda, PhD • *University of Pennsylvania*

11:55 AM

Alternatively Activated Macrophage Secretome Modulates Metastatic Potential of Ovarian Cancer Cells
Kaitlin Fogg, PhD • *University of Wisconsin - Madison*

12:10 PM –1:35PM — **Lunch Break** HUB Ballroom (Husky Union Building)

12:10 PM –1:15PM — **SUMMIT: Measuring the Success and Impact of Ovarian Cancer Nonprofit Research Funding** HUB 214 (Limited Seating)

Except where otherwise noted, all oral presentation sessions will be in Kane Hall.

SESSION 4 — NOVEL THERAPEUTICS: RESPONSE AND RESISTANCE OF OVARIAN CANCER – KANE HALL

1:35 PM — INVITED SPEAKER PRESENTATION

Novel combination strategies for recurrent ovarian cancer
Ursula Matulonis, MD • *Dana Farber Cancer Institute, Harvard Medical School*

2:15 PM — INVITED SPEAKER PRESENTATION

Cell Cycle Checkpoints as Therapeutic Targets
Jung-Min Lee, MD • *National Cancer Institute*

ABSTRACT SESSION

2:55 PM

Total Abdominal Ultra-Rapid FLASH Irradiation Demonstrates Decreased Gastrointestinal Toxicity Compared to Conventional Total Abdominal Irradiation in Mice
Karen Levy, MD • *Stanford University*

3:10 PM

Cancer Stem Cells: Distinct Seeds for Recurrent Ovarian Cancer
Nuzhat Ahmed, PhD • *Fiona Elsey Cancer Research Institute (Australia)*

3:25 PM

A Phase 2 Study of Tpiv200/Hufr-1 (A Multi-Epitope Folate Receptor Alpha Vaccine) in Combination with Durvalumab in Patients with Platinum Resistant Ovarian Cancer
Dmitriy Zamarin, MD, PhD • *Memorial Sloan Kettering Cancer Center*

3:40 PM — **BREAK**

ABSTRACT SESSION CONTINUED

4:00 PM

Antitumor Response After Vaccination Targeting Insulin Growth Factor Binding Protein 2 in a Syngeneic Mouse Model of Ovarian Cancer Localized by Three-Dimensional In Vivo Optical Imaging
Denise Cecil, MD, PhD • *University of Washington*

4:15 PM

A Single Cell RNA-Sequencing Approach to Uncovering Human Ovarian Tumor and Immune Cell Heterogeneity, and Their Response to Mullerian Inhibiting Substance Using Patient Ascites Samples
David Pepin, PhD • *Massachussetts General Hospital, Harvard Medical School*

4:30 PM

BRCA1 Intron Retention Generates Truncated Proteins that Avoid BRCT Mutation Misfolding and Promote PARP Inhibitor Resistance
Neil Johnson, PhD • *Fox Chase Cancer Center*

4:45 PM

ATR Inhibition Targets Treatment Resistant Ovarian Cancer
Erin George, MD • *University of Pennsylvania*

5:00 PM

The Role of NFAT3 in Ovarian Cancer Quiescence and Chemotherapy Resistance
Alex Cole, PhD • *University of Michigan*

5:15 PM — **Wrap Up**

Please see the walking route on the back cover.

CONTINUING MEDICAL EDUCATION (CME)

ACCREDITATION STATEMENT

This activity has been planned and implemented in accordance with the accreditation requirements of the Accreditation Council for Continuing Medical Education (ACCME) through the joint providership of The American Association for Cancer Research (AACR) and the Rivkin Center for Ovarian Cancer. The American Association for Cancer Research (AACR) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education (CME) activities for physicians.

CREDIT DESIGNATION STATEMENT

AACR has designated this live activity for a maximum of 16.5 ***AMA PRA Category 1 Credit(s)™.*** Physicians should only claim credit commensurate with the extent of their participation in the activity.

Credit certification for individual sessions may vary, dependent upon compliance with the ACCME Accreditation Criteria. The final number of credits may vary from the maximum number indicated above.

CLAIMING CME CREDIT

Physicians and other health care professionals seeking AMA PRA Category 1 Credit(s)TM for this live continuing medical education activity must complete the online CME Request for Credit Survey by **Monday, October 29, 2018**. Certificates will only be issued to those who complete the survey. The Request for Credit Survey will be available via a link on the 12th Biennial Ovarian Cancer Research Symposium website or via email. ***Your CME certificate will be sent to you via email after the completion of the activity.***

STATEMENT OF EDUCATIONAL NEED

Approximately 22,000 women in the United States are diagnosed with ovarian cancer every year. The overall 5-year survival rate is only 46% for ovarian cancer, and the rate is markedly lower for advanced stage cancers. Sadly, the survival rates have only improved slightly over 40 years. While ovarian cancer is the most deadly gynecologic cancer, it is underrepresented in both research funding and research participants. Additionally, complicating the difficulty in diagnosing the disease beyond its physical complexities is that ovarian cancer is a rare disease and many providers will have limited access to information on the signs, symptoms, and latest techniques to diagnose the disease.

In this course, we are addressing major needs in ovarian cancer care and research by educating health care providers and researchers in the latest research on improving treatment, early detection, and prevention, as well as promoting a better understanding of the many diseases under the umbrella of ovarian cancer.

The Planning Committee has designed this event to benefit the following audiences: basic scientists, epidemiologists, clinical scientists, and clinicians as well as geneticists, public health researchers, nurses, and advocates for ovarian cancer research. This conference encourages the collaboration between professionals with a wide variety of expertise including: gynecologic oncology, medical oncology, pathology, genetics, molecular biology, cell biology, public health, behavioral epidemiology, translational research, patient care, and patient advocacy.

After participating in this CME activity, physicians should be able to:

- Define cancer control strategies to detect ovarian cancer at the earliest stages and to prevent the disease
- Articulate the molecular mechanisms and genomic drivers of ovarian cancer
- Explain the elements of tumor microenvironment and immunology as they contribute to ovarian cancer growth and as possible targets for treatment
- Identify targeted therapies against ovarian cancer and assess the response of new therapies and lack of response in cases of recurrence and therapy resistance.

DISCLOSURE STATEMENT

It is the policy of the AACR that the information presented at AACR CME activities will be unbiased and based on scientific evidence. To help participants make judgments about the presence of bias, AACR will provide information that Scientific Program Committee members and speakers have disclosed about financial relationships they have with commercial entities that produce or market products or services related to the content of this CME activity. This disclosure information will be made available in the ***Program/Proceedings*** of this conference.

ACKNOWLEDGEMENT OF FINANCIAL OR OTHER SUPPORT

This activity is supported by Professional Educational Grants which will be disclosed at the activity.

QUESTIONS ABOUT CME?

Please contact the Office of CME at (215) 440-9300 or cme@aacr.org.

DISCLOSURE OF FINANCIAL RELATIONSHIPS

In compliance with the standards set by the Accreditation Council for Continuing Medical Education (ACCME), it is the policy of the American Association for Cancer Research (AACR) that the information presented at CME activities will be unbiased and based on scientific evidence. To help participants make judgments about the presence of bias, the AACR has provided information that planning committee members, speakers, and abstract presenters have disclosed about financial relationships they have with commercial entities that produce or market products or services related to the content of this CME activity.

Relationships are abbreviated as follows: E, Employee of listed company, G, Grant/research support recipient, A, Advisor or review panel member, C, Consultant, S, Stock Shareholder, SB, Speakers' Bureau, H, Honoraria, O, Other.

LAST NAME	FIRST NAME	COMPANY	RELATIONSHIPS	TYPE	ROLE
Ahmed	Nuzhat	Gynecological Cancer Research Ctr.	No Relationships		Speaker
Ailles	Laurie	University Health Network	No Relationships		Speaker
Balkwill	Frances	Barts Cancer Inst.	Inflazome	C	Program Committee, Speaker
Barger	Carter	UNMC	No Relationships		Speaker
Baron	Amy	American Association for Cancer Research	No Relationships		Program Committee
Barton	Jennifer	University Of Arizona	Nikon Corporation	G	Speaker
Bernstein	Kara	University of Pittsburgh School of Medic	No Relationships		Speaker
Brenton	James	University of Cambridge	Inivata Ltd., AZ, Aprea	G,H,S	Speaker
Burdette	Joanna	Univ. of Illinois at Chicago	No Relationships		Speaker
Campagnola	Paul	University of Wisconsin- Madison	No Relationships		Speaker
Cecil	Denise	Univ. of Washington	No Relationships		Speaker
Chien	Jeremy	The Univ. of New Mexico	No Relationships		Speaker
Cho	Kathleen	Univ. of Michigan Medical School	No Relationships		Speaker
Cole	Alex	University of Michigan	No Relationships		Speaker
Corona	Rosario	Cedars-Sinai Medical Center	No Relationships		Speaker
Crase	Jamie	Univ. of Washington	No Relationships		Speaker
Crum	Christopher	Brigham & Women's Hospital	No Relationships		Speaker
D'Andrea	Alan	Dana-Farber Cancer Inst.	Lilly Oncology, EMD Serono, Intelliq, Sierra Oncology, Formation Biologics, Indeaya Inc., Cedilla Therapeutics	L, S	Speaker
Dhillon	Kiran	Rivkin Center for Ovarian Cancer Research	No Relationships		Program Committee
Disis	Mary	Univ. of Washington	Celgene, EMD Serono, Pfizer, Epithany, Janssen	G,S	Program Committee, Speaker
Farkkila	Anniina	Dana-Farber Cancer Inst.	No Relationships		Speaker
Fogg	Kaitlin	University of Wisconsin - Madison	No Relationships		Speaker
Fuh	Katherine	Washington Univ. School of Medicine	No Relationships		Speaker
George	Erin	Univ. of Pennsylvania Hospital	No Relationships		Speaker
Heinzl	Nicole	Univ. of Michigan	No Relationships		Speaker
Heydrich	Carmelina	Color Genomics, Inc.	Color Genomics Inc.	E	Speaker
Hill	Sarah	Dana-Farber Cancer Inst.	No Relationships		Speaker
Hooda	Jagmohan	Univ. of Pennsylvania	No Relationships		Speaker
Johnson	Neil	Fox Chase Cancer Ctr.	No Relationships		Speaker
King	Mary-Claire	Univ. of Washington School of Medicine	No Relationships		Speaker
Lee	Jung-Min	National Cancer Institute	No Relationships		Speaker
Lengyel	Ernst	Univ. of Chicago	No Relationships		Speaker
Levine	Douglas	New York University	Clovis, Tesaro, Merck, mTrap Inc.	A	Speaker
Levy	Karen	Stanford Univ.	No Relationships		Speaker
Manchanda	Ranjit	Barts Cancer Institute	No Relationships		Speaker
Matulonis	Ursula	Dana-Farber Cancer Inst.	2X Oncololgy, Merck, Mersana, FujiFilm, Geneos, Immunogen	A,S	Speaker
Michels	Kara	National Cancer Institute	No Relationships		Speaker
Nelson	Brad	BC Cancer Agency	No Relationships		Speaker
Ohashi	Pamela	Princess Margaret Cancer Centre	No Relationships		Program Committee, Speaker
Paul	Marcie	Ovarian Cancer Research Fund Alliance	No Relationships		Speaker
Pearce	Celeste	Univ. of Michigan School of Public Health	No Relationships		Speaker
Pepin	David	MGH/Harvard Medical School	No Relationships		Speaker
Risques	Rosa Ana	Univ. of Washington	TwinStrand Biosciences	G	Speaker
Rivkin	Saul	Rivkin Center for Ovarian Cancer Research	No Relationships		Speaker
Skubitz	Amy	University of Minnesota	No Relationships		Speaker
Soong	Thing	Univ. of Washington	No Relationships		Speaker
Stover	Elizabeth	Dana-Farber Cancer Institute	No Relationships		Speaker
Swisher	Elizabeth	Univ. of Washington	No Relationships		Program Committee, Speaker
Wen	Yun-Fei	UT MD Anderson Cancer Ctr.	No Relationships		Speaker
Wu	Rong	Univ. of Michigan	No Relationships		Speaker
Zamarin	Dmitriy	Mem. Sloan Kettering Cancer Ctr.	Merck, Synlogic, Hookipa Biotechnologies	C	Speaker
Zanivan	Sara	Beatson Inst. for Cancer Research	No Relationships		Speaker
Zhang	Shuang	NYU Langone Medical Center	No Relationships		Speaker

CME FAQS

THE RIVKIN CENTER FOR OVARIAN CANCER & THE AMERICAN ASSOCIATION FOR CANCER RESEARCH PRESENT THE 12TH BIENNIAL OVARIAN CANCER RESEARCH SYMPOSIUM

September 13 – 15, 2018 • Seattle, WA

The American Association for Cancer Research (AACR) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education activities for physicians.

Co-sponsored by the Rivkin Center, AACR has designated this live activity for a maximum of 16.5 ***AMA PRA Category 1 Credit(s)™.*** Physicians should only claim credit commensurate with the extent of their participation in the activity.

Other healthcare professionals, such as nurses, pharmacists, and physician-assistants, may be able to use their participation at CME-designated activities to satisfy their licensing requirements. See below for more information on how to request a Certificate of Participation.

HOW DO ATTENDEES APPLY FOR CREDIT?

All individuals requesting continuing medical education credits must complete a CME Request for Credit Survey that will be sent via e-mail to all attendees and will also be available online at the CME tab of the symposium website.

WHO CAN RECEIVE CME CREDITS FOR ATTENDING THE MEETING?

Physicians (MD and DO) can receive a CME Certificate by submitting a CME Request for Credit Survey.

Non-physicians may request a Certificate of Participation by completing a CME Request for Credit. Participation hours in this educational event may satisfy licensing requirements for certain specialties.

WHAT IS THE DEADLINE FOR SUBMITTING A CREDIT SURVEY?

Evaluation surveys and CME credit requests must be received by Monday, October 29th, 2018.

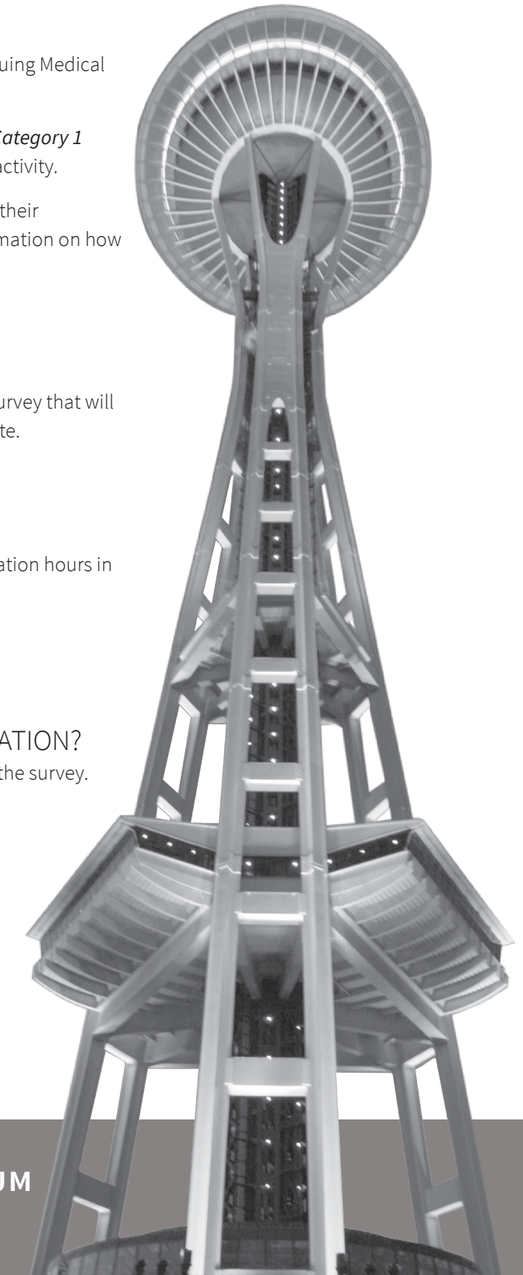
WHEN WILL I RECEIVE MY CME CERTIFICATE OR MY CERTIFICATE OF PARTICIPATION?

Certificates will be processed and e-mailed by Monday, October 29th, 2018 for those who have completed the survey.

QUESTIONS ABOUT CME?

Please contact the AACR Office of CME at (215) 440-9300, cme@aacr.org, or see the CME staff onsite at the conference for assistance.

Ron Arthur
Assistant Director, Office of CME
Phone: (215) 446-7139
E-mail: ronald.arthur@aacr.org



THE RIVKIN CENTER FOR OVARIAN CANCER & THE AMERICAN ASSOCIATION FOR CANCER RESEARCH GRATEFULLY ACKNOWLEDGE THE MANY SUPPORTERS OF THIS CONFERENCE:

(As of August 25th, 2018, this is the most complete list available at the time of printing. Appropriate acknowledgment will be given to all supporters on the day of the event.)

PROFESSIONAL EDUCATION GRANTS:

Tesaro
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NATIONAL OVARIAN CANCER ACADEMY

The Rivkin Center for Ovarian Cancer thanks the DOD-Sponsored National Ovarian Cancer Academy for the Academy's continued participation in the organization of the Ovarian Cancer Research Symposium and contributions to the understanding of ovarian cancer.

DoD OCA | OVARIAN CANCER ACADEMY

A NOTE FROM THE ACADEMY:

2018 DOD-SPONSORED NATIONAL OVARIAN CANCER ACADEMY

Our ability to truly impact the lives of women diagnosed with ovarian cancer is dependent on our ability to discover new ways of diagnosing and treating this disease. Our slow progress on this front has as much to do with the culture of biomedical research as it does with the complexity of this disease. Recognizing the need for innovation in this area, in 2009 the DOD's Ovarian Cancer Research Program initiated a virtual national training program to support the career development of early career investigators committed to the study of this disease. Since that time, 8 new investigators have graduated from the DOD-sponsored Ovarian Cancer Academy (OCA) – a novel training program designed to build a community of compassionate scientists and physicians who have acquired the skills necessary to effectively collaborate, as a team, working toward the common goal of eradicating this disease. OCA Scholars work in solidarity with ovarian cancer patients, so that their important perspectives can be incorporated into the research process – sharing their ideas and passion toward the common goal of overcoming this disease. Please read more about this program at: *http://www.ovariancanceracademy.org*.

The leadership of this year's OCA (Nita J. Maihle, Dean, Douglas Levine, Assistant Dean, Karen Wylie, Science Officer), the OCA Scholars (Rebecca Arend, Juan Cubillos-Ruiz, Sophia George, Yang Yang Hartwich, Neil Johnson , John Liao, Karen McLean, Geeta Mehta, Anirban Mitra, David Pepin, Erinn Rankin, Xian Wa, and Dmitriy Zamarin), and their mentors (Carol Aghajanian, Mike Birrer, Jeff Boyd, Ron Buckanovich, Jonathan Berek, Kathy Cho, Jose Conejo-Garcia, Chris Crum, Nora Disis, Patricia Donohoe, Ken Nephew, Alessandro. Santin, and Brian Slomowitz) are grateful to the Rivkin Center for their leadership in the vital field of ovarian cancer research, as well as for the opportunity to participate in this year's 12th Biennial Ovarian Cancer Research Symposium.

AACR FUNDED SCHOLAR-IN-TRAINING AWARDS

Five presenters of meritorious abstracts have been selected by the Symposium Chairpersons to receive awards to attend this conference. All graduate and medical students, postdoctoral fellows, and physicians-in training who are AACR Associate members and submitted an application were eligible for consideration. The names of the Scholar-in-Training awardees, their affiliations, and the proffered presentation date/time or poster number are provided below.

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AACR SCHOLAR-IN-TRAINING AWARDS

Rosario Corona, PhD
Cedars-Sinai Medical Center
Los Angeles, California, USA
Abstract Presentation
Friday, September 14, 2018, 3:25pm

Erika Dahl
Penn State College of Medicine
Hershey, Pennsylvania, USA
Poster GMM-022
Friday, September 14, 2018, 6:30pm-8:15pm

Kara Michels
National Cancer Institute
Bethesda, Maryland, USA
Abstract Presentation
Friday, September 14, 2018, 11:25pm

Venkatesh Krishnan
Stanford University
Palo Alto, California, USA
Poster TMIM-076
Friday, September 14, 2018, 6:30pm-8:15pm

Alexandria Young
University of Illinois at Chicago
Chicago, Illinois, USA
Poster NT-119
Thursday, September 13, 2018, 6:30pm-8:15pm

JOIN US FOR OUR SPECIAL SESSIONS DURING LUNCH

MEET THE EXPERTS ROUNDTABLE DISCUSSIONS

Friday, September 14, 2018, 12:10pm to 1:20pm | HUB Ballroom

Roundtable discussions on hot topics in ovarian cancer led by experts! Sign-up by Registration/Check-in Thursday, Sept. 13 or Friday, Sept. 14 (before lunch). Limited to availability and only one table sign-up per attendee.

DISCUSSION TOPICS AND LEADERS:

STATE OF EARLY DETECTION OF OVARIAN CANCER
Charles Drescher, MD (Swedish Cancer Institute/Fred Hutch)
Yang Yang Hartwich, PhD (Yale University)

IMMUNOTHERAPY FOR OVARIAN CANCER
Nora Disis, MD (University of Washington/Fred Hutch)
Juan Ruiz, PhD (Weill Cornell Medical College)

ROLE OF MICROENVIRONMENT IN OVARIAN CANCER
Pamela Ohashi, PhD (Princess Margaret Cancer Centre)
Anirban Mitra, PhD (Indiana University School of Medicine)

PARP INHIBITORS AND RESISTANCE MECHANISMS
Alan D'Andrea, MD (Dana-Farber Cancer Institute)
Neil Johnson, PhD (Fox Chase Cancer Center)

FUTURE OF OVARIAN CANCER THERAPIES
Ursula Matulonis, MD (Dana-Farber Cancer Institute)
Wa Xian, PhD (University of Texas, Houston)

COMMUNICATING SCIENCE TO THE GENERAL PUBLIC
Frances Balkwill, PhD (Barts Cancer Institute)
Dmitriy Zamarin, MD/PhD (Memorial Sloan Kettering Cancer Center)

GENETIC TESTING: IMPLICATIONS FOR PREVENTION AND TREATMENT
Elizabeth Swisher, MD (University of Washington)
Ranjit Manchanda, PhD (Barts Cancer Institute)

SUMMIT: MEASURING THE SUCCESS AND IMPACT OF OVARIAN CANCER NONPROFIT RESEARCH FUNDING

Saturday, September 15, 2018, 12:10pm to 1:15pm | HUB 214

Representatives from several nonprofit organizations that fund ovarian cancer research will present their approaches to measuring the success and impact of their funded grants, followed by discussion. This session has limited seating.

PRESENTERS:

Powel Crosely (Granulosa Cell Tumour Research Foundation)

Kiran Dhillon, PhD (Rivkin Center for Ovarian Cancer)

Susan Leighton (Ovarian Cancer Research Funding Alliance)

Adoracion Pegalajar-Jurado (OvaCure)

Rivkin Center for Ovarian Cancer

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SESSION 1: DETECTION & PREVENTION OF OVARIAN CANCER

INVITED SPEAKERS

ABSTRACT PRESENTATIONS

40 **POSTER DP–001 | SESSION I: THURSDAY**
Key achievement of the fast Pade transform in magnetic resonance spectroscopy for early ovarian cancer diagnostics
Karen Belkic, MD
Karolinska Institute (Sweden)

41 **POSTER DP–002 | SESSION II: FRIDAY**
Comparison of potential ovarian cancer biomarkers by mass spectrometry-based proteomic analysis of residual Pap test fluid, cervical swabs, and tumor tissue from an ovarian cancer patient
Kristin L. M. Boylan, PhD
University of Minnesota

42 **POSTER DP–003 | SESSION I: THURSDAY**
Toward a hematopoietic stem cell-based prophylactic immuno-gene therapy approach for ovarian cancer
Chang Li, PhD
University of Washington

43 **POSTER DP–004 | SESSION II: FRIDAY**
Describing the odds of long-term survival among women diagnosed with high-grade serous ovarian cancer using electronic medical records
Christina L. Clarke, MS
Kaiser Permanente Colorado

44 **POSTER DP–005 | SESSION I: THURSDAY**
RNA-sequencing of Fallopian tube – Fimbria and HGSC from BRCA mutation carriers
Sophia HL George, PhD
Leonard Miller School of Medicine, Sylvester Comprehensive Cancer Center

45 **POSTER DP–006 | SESSION II: FRIDAY**
The effect of age and OCP use on the incidence of pre-cancerous p53 lesions and the development of high grade serous ovarian carcinoma
Kendall Greening, MSc
University of British Columbia (Canada)

46 **POSTER DP–007 | SESSION I: THURSDAY**
Polycystic ovary syndrome and ovarian cancer risk: A Mendelian randomization analysis
Holly R. Harris, DSc
Fred Hutchinson Cancer Research Center

47 **POSTER DP–008 | SESSION II: FRIDAY**
Silica gel encapsulation as a potential tool to identify dormant and drug-resistant cell populations in ovarian cancer
Tiffany Lam, BS
University of Minnesota Twin Cities

48 **POSTER DP–009 | SESSION I: THURSDAY**
Full-term births are protective for ovarian cancer – what about incomplete pregnancies?
Alice W. Lee, PhD
California State University, Fullerton

49 **POSTER DP–0010 | SESSION II: FRIDAY**
Intraperitoneal Chemotherapy Use and Outcomes: Exploration of UPMC Ovarian Cancer Registry Data
Faina Linkov, PhD
University of Pittsburgh School of Medicine

50 **POSTER DP–0011 | SESSION II: FRIDAY**
Pre-diagnosis use of menopausal hormone therapy associated with better ovarian cancer survival
Celeste L. Pearce, PhD
University of Michigan, Ann Arbor

51 **POSTER DP–0012 | SESSION II: FRIDAY**
Studying the signaling pathways of distinct subpopulations of cells generated upon PTEN deletion from FTE and identification of markers of early tumorigenesis
Angela Russo, PhD
University of Illinois at Chicago

52 **POSTER DP–013 | SESSION I: THURSDAY**
Development and validation of circulating CA125 prediction model in postmenopausal women without ovarian cancer
Naoko Sasamoto, MD, MPH
Brigham and Women's Hospital

53 **POSTER DP–014 | SESSION II: FRIDAY**
Lifetime number of ovulatory cycles are differentially associated with ovarian cancer histotypes: an analysis from the Ovarian Cancer Cohort Consortium (OC3)
Britton Trabert, PhD
National Cancer Institute

54 **POSTER DP–015 | SESSION I: THURSDAY**
Biotinylated Estrogens a Novel Tool for Early Detection of Adduct In Ovarian Cancer
Kaushlendra Tripathi, PhD, MS
UAB and Mitchell Cancer Institute

Rivkin Center for Ovarian Cancer

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POPULATION TESTING FOR OVARIAN CANCER GENE MUTATIONS FOR PRIMARY PREVENTION

Ranjit Manchanda, MD/PhD

Barts Cancer Institute, London, UK

No abstract provided.

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OVARIAN CANCER DETECTION USING ULTRA-SENSITIVE SEQUENCING: CHALLENGES AND OPPORTUNITIES

Rosana Risques, PhD

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No abstract provided.

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LINEAGE CONTINUITY BETWEEN EARLY TUBAL SEROUS PROLIFERATIONS (ESPS/STILS) AND DISSEMINATED HIGH-GRADE SEROUS CARCINOMAS: A MODEL FOR "PRECURSOR ESCAPE"

Thing Rinda Soong, MD, PhD, MPH[1], Brooke E. Howitt, MD[2], Alexander Miron, PhD[3], Neil Horowitz, MD[4], Frank Campbell, PhD[3], Colleen M. Feltmate, MD[4], Michael G. Muto, MD[4], Ross S. Berkowitz, MD[4], Marisa R. Nucci, MD[5], Wa Xian, PhD[6], Christopher P. Crum, MD[5]

[1]Department of Pathology, University of Washington Medical Center, Seattle, WA; [2]Department of Pathology, Stanford University Medical Center, Palo Alto, CA; [3]Plexseq Diagnostics, Cleveland, Ohio; [4]Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Boston MA; [5]Division of Women's and Perinatal Pathology, Department of Pathology, Brigham and Women's Hospital, Boston, MA; [6]Institute of Molecular Medicine, University of Texas Health Sciences Center, Houston, TX

It is now well known that the distal fallopian tube is a site of origin for many high-grade serous carcinomas (HGSCs). HGSCs are thought to arise via intra-peritoneal spread of tumor cells from serous tubal intra-epithelial carcinomas (STICs) that share identical *TP53* mutations. However, a dualistic model of pathogenesis might be indicated because many HGSCs are not associated with STICs and their site(s) of origin is unclear. This fact has important implications for both screening and cancer preventive strategies. In addition to STICs, the tube also hosts *TP53* mutation-positive early serous proliferations (ESPs) that comprise a morphologic spectrum ranging from p53 signatures to serous tubal intraepithelial lesions (STILs). ESPs are more common than STICs and are not considered malignant; however, they are often the only abnormality found in fallopian tubes of women with HGSCs. The purpose of this study was to determine if a genetic relationship exists between isolated ESPs and concurrent HGSCs in the absence of STIC. Fallopian tubes from 32 HGSCs without a co-existing STIC on initial review were exhaustively and serially sectioned. The presence of either occult STIC or ESP in serial sections was documented and DNAs from tissues containing ESPs, cancers and normal control epithelia were interrogated for *TP53* mutations by targeted amplicon-based sequencing with average coverage reads >4000 across DNA replicate samples. Serial sectioning unearthed a STIC in 3 of 32 (9.3%) and ESPs in 12 (37.5%). Four of 12 ESPs (33.0%) shared an identical *TP53* mutation with the concurrent cancer at an allele frequency ≥5%. An additional 5 ESPs (41.7%) also shared identical *TP53* mutations with concurrent cancers at a lower (<5%) allele frequency. All control epithelia were *TP53* mutation-negative. This study for the first time indicates lineage identity between ESPs in the distal tube and some metastatic HGSCs via a shared site-specific *TP53* mutation. It supports a novel carcinogenic sequence in which a proliferation that does not fulfill the criteria for STIC (such as a p53 signature or STIL) could eventually culminate in a metastatic serous cancer via a phenomenon we term "precursor escape." This paradigm for cancer development underscores the likelihood that multiple precursor types in the fallopian tube could ultimately contribute to the development of serous cancer, and would explain the apparent sudden onset of cancers without co-existing STICs as well as the emergence of "primary peritoneal" carcinomas years after removal of benign-appearing ovaries and fallopian tubes. Moreover, it parallels observations made on the clonal nature of multi-site endometriosis and introduces a similar and novel precancer-cancer paradigm for HGSCs where precursor initiation and progression to malignancy are separated spatially and temporally.

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EXPOSING THE LOCAL FACTORS THAT CONTRIBUTE TO OVARIAN COLONIZATION OF FALLOPIAN TUBE-DERIVED TUMORS

Joanna E. Burdette, Katherine Zink, Matthew Dean, and Laura M. Sanchez

University of Illinois-Chicago

High grade serous cancer (HGSC) originates in the fallopian tube and typically invade and colonize the ovary as part of peritoneal metastasis. The purpose of this study was to define the factors that contribute to ovarian specific metastasis of fallopian tube HGSC. While protein factors have been explored that facilitate migration and invasion of fallopian tube cell to the ovary, the small molecule communication between the tissues during metastasis remains largely unexplored. Due to the proximity of ovarian primary metastasis, an experimental design was optimized using imaging mass spectrometry to capture the spatial composition of small molecules uniquely expressed when fallopian tube-derived tumor cells were grown in the microenvironment of the ovary as a model of primary metastasis. The observed mass-to-charge ratios (m/z's) that were induced specifically in co-culture represent small molecules that may contribute to the metastasis of HGSOC selectively to the ovary. Human fallopian tube epithelial HGSOC and tumorigenic murine oviductal epithelial cells, but not normal cell types, repeatedly induced a signal from the ovary at m/z 170. This signal was identified as norepinephrine, which was confirmed to simulate invasion of ovarian cancer cells lacking wildtype p53. Norepinephrine has an established role in ovarian cancer metastasis to secondary sites and the use of beta-blockers in women is associated with increased survival. The use of imaging mass spectrometry also revealed a series of new molecules that are specifically enhanced from the ovary or the fallopian tube during co-culture. These molecules may reveal pathways that contribute to metastasis and biological targets for therapeutic intervention to block ovarian metastasis of fallopian tube-derived HGSOC. The developed mass spectrometry method can be adapted to other mammalian-based model systems for investigation of untargeted metabolomics that facilitate metastasis.

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NOTES

DISTINCT CELL/TISSUE SOURCES OF HIGH-GRADE SEROUS OVARIAN CANCER

Shuang Zhang, Benjamin G. Neel

Laura and Isaac Perlmutter Cancer Center, NYU-Langone Medical Center, NY 10016, USA

The cell-of-origin of high grade serous ovarian carcinoma (HGSC) has been a focus of debate. Here, by using genetic mouse models as well as cognate organoid systems, we assessed the tumor forming capacity and properties of the fallopian tube epithelium (FTE) and ovarian surface epithelium (OSE) bearing the same oncogenic abnormalities. Combined RB family inactivation (via T121 expression) and Tp53 mutation in Pax8+ fallopian tube secretory cells causes transformation and characteristics of Serous Tubal Intraepithelial Carcinoma (STIC). This genetically engineered mouse HGSC model is faithfully recapitulated in fallopian organoids, from which serous ovarian cancer with broad abdominal metastasis is generated upon orthotropic injection. The same genetic events in Lgr5+ OSE cells organdies derived from these cells also result in an apparent neoplastic process, expressing markers of early serous carcinoma (but not Pax8), which subsequently develop into serous-like tumors. Hence, both Pax8+ fallopian tube epithelial cells and Lgr5+ ovarian surface epithelial cells can undergo similar neoplastic transformation, suggesting that HGSC might derive from distinct cell and tissue sources. Similar organoid systems can be used to rapidly model other gene combinations associated with HGSC.

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INCREASING ACCESS TO GENETIC TESTING FOR HEREDITARY BREAST AND OVARIAN CANCER: EXPERIENCE FROM HIGH-VOLUME OB/GYN CLINIC IN TEXAS

Carmelina Heydrich, Lily K Servais, Sarah A Popelka, Brian K Moreau, Katie N Allio, Anjali D Zimmer

Color Genomics, Burlingame, CA, USA

INTRODUCTION: Currently most genetic testing for hereditary cancer risk is performed on individuals with strong personal and/or family histories of cancer. However, as much as 50% of people with a BRCA1 or BRCA2 gene mutation may not have significant family history. As a result, many people are often not identified as having a pathogenic mutation until after they or someone in their family has already been diagnosed with cancer. This study aims to provide insights into prevention of hereditary breast and ovarian cancer by providing broad access to testing in the ob/gyn care setting.

METHODS: We describe the demographics and genetic results of 1,113 individuals who were referred by a physician to receive genetic testing for hereditary cancer risk from Color Genomics. The test is a next generation sequencing (NGS) based assessment of 30 genes associated with hereditary cancer risk. The physicians offered the Color Test to their patients at their wellness visit and provided education about genetic testing via online newsletter to inform patients and increase interest for the program. The OB/GYN practice consists of 22 clinicians and serves almost 50 thousand patients.

RESULTS: More than 48,253 patients were sent information about this genetic testing via email. The email open rate was 39.6%. 1,113 individuals underwent testing. In the cohort, a total of 90 pathogenic or likely pathogenic variants were identified for an overall pathogenic rate of 8.1%. Twenty individuals (1.8%) were identified as having mutations in genes that increased risk of ovarian cancer (BRCA1, BRCA2, BRIP1, EPCAM, MLH1, MSH2, MSH6, PALB2, PMS2, RAD51C, RAD51D, STK11 and TP53). The average age of the participant who underwent testing was 44.4 years of age. The cohort was 44% Caucasian. Nineteen patients reported Ashkenazi Jewish (AJ) ancestry; the pathogenic rate among people who indicated AJ ancestry was 21%. Health history was self-reported and 47 individuals reported a history of cancer. Only two individuals reported a personal history of ovarian cancer--both were negative for any pathogenic mutations.

CONCLUSIONS: The cohort analyzed here included a broader population than has historically qualified for or been offered genetic testing for hereditary cancer risk. The approach taken by this OB/GYN practice highlights a novel way to identify people at increased risk for cancer due to inherited mutation. The practice identified 90 patients who were at increased risk for cancer, including 20 women who were at increased risk for ovarian cancer—none of whom were yet affected—highlighting the opportunity for prevention of ovarian cancer as well as other cancers.

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OVARIAN CANCER-ASSOCIATED RAD51D MUTATIONS WHICH IMPAIR ITS INTERACTION WITH XRCC2 RESULT IN DNA REPAIR DEFICIENCY

Robert A. Baldock[1], Catherine A. Pressimone[1], Jared M. Baird[1], Anton Y. Khodakov[1], Yoav Karpenshif[1], Edwige B. Garcin[2], Stéphanie Gon[2], Mauro Modesti[2], **Kara A. Bernstein**[1]

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The proficiency of ovarian cancer cells to repair DNA double-strand breaks (DSBs) by homologous recombination (HR) is a key determinant in predicting response to targeted therapies such as PARP inhibitors (PARPi). The RAD51 paralogs act downstream of BRCA1/2 to facilitate HR. Numerous epidemiological studies have linked mutations in the RAD51 paralogs with hereditary ovarian cancer predisposition. Despite their substantial links to cancer predisposition and development, RAD51 paralog function during HR has remained elusive, in part due to limitations in studying recombination events downstream of RAD51 filament formation. Here we investigate the impact of cancer-associated mutations in the RAD51 paralog, RAD51D, using yeast 2/3-hybrid assays to screen for altered protein-protein interactions. Following the identification of mutations that disrupt the interaction between RAD51D and XRCC2 in yeast, we validated the interaction by co-immunoprecipitation in human cells. Importantly, we determined the impact of these mutations on HR-proficiency using a direct-repeat recombination assay. By characterizing the impact of cancer-associated mutations in the RAD51 paralogs on HR-proficiency, we aim to develop more effective predictive models for therapeutic sensitivity and resistance in patients who harbor similar mutations in these essential genes.

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METABOLIC SYNDROME AND RISK OF OVARIAN CANCER IN THE UNITED STATES: AN ANALYSIS OF LINKED SEER–MEDICARE DATA

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BACKGROUND: Ovarian cancer is typically diagnosed at later stages and we have much to learn regarding its etiology. Identifying modifiable risk factors has the potential to reduce the burden of ovarian cancer. Metabolic dysregulation is thought to play a role in the development of this disease, but associations between risk of ovarian cancer and markers of metabolic dysregulation are inconsistent across studies (e.g., diagnoses of metabolic syndrome or diabetes, measurement of circulating lipids). Many studies are not able to evaluate associations across subtypes of ovarian cancer, which is crucial given our knowledge about the heterogeneous etiologies of ovarian cancer histotypes.

PURPOSE: Using data from U.S. women who were eligible for Medicare, we estimated risk of ovarian cancer associated with metabolic syndrome and its components, as documented in medical records. We also determined if these associations differed by histotype.

METHODS: We created a case-control study within the Surveillance, Epidemiology and End Results (SEER)–Medicare linked database. Cases (n = 16,850) were women diagnosed with epithelial ovarian or fallopian tube cancer between ages 70 to 90 from 1994 through 2013. Controls (n = 281,878) were a 5% sample of female Medicare enrollees residing in the same SEER registry area as cases. Cases and controls were required to be enrolled in Medicare for at least 1 year continuously during the 2 to 3 years before an index date (date of diagnosis for cases, randomly selected date for controls). We examined whether factors related to metabolic syndrome (obesity, fasting glucose, high blood pressure, HDL cholesterol, triglycerides), individually or combined (using several standard definitions of metabolic syndrome), were associated with ovarian cancer. These factors were defined using ICD-9-CM codes from inpatient/outpatient diagnoses during the 2 to 5 years before the index dates. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression adjusted for index date, age, race, registry, smoking status, and state buy-in status.

RESULTS: Medical record documentation of high blood pressure (OR 1.06, 95%CI 1.03–1.10) and high triglycerides (OR 1.11, 95%CI 1.07–1.15) were associated with modestly increased risk of ovarian cancer. Metabolic syndrome was not associated with risk overall. High blood pressure increased risk of endometrioid (OR 1.19, 95%CI 1.05–1.35) and clear cell tumors (OR 1.25, 95% CI 1.01–1.55). High triglycerides consistently increased risk across histotype and grade. We identified reduced risks for serous cancer with high fasting glucose (OR 0.88, 95% CI 0.83–0.94); this association strengthened when limiting to high-grade serous tumors (OR 0.80, 95% CI 0.74–0.86).

CONCLUSIONS: Our data suggest that individual components of metabolic syndrome are associated with ovarian cancer, rather than metabolic syndrome itself. For serous cancers, we saw associations in different directions for elevated triglycerides and fasting glucose—indicating that evaluating metabolic syndrome as a composite outcome could be misleading and etiologically uninformative in ovarian cancer studies. While both lipid and glucose metabolism can be dysregulated in metabolic syndrome, the consequences of this dysregulation likely influence the development of serous ovarian cancers via different mechanisms.

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PROTEIN AND IMAGING MARKERS FOR EARLY DETECTION OF EPITHELIAL OVARIAN CANCER

Jennifer K. Barton[1], Anna E. Lokshin[2], David A. Fishman[3], John F. Black[4]

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A central problem in ovarian cancer is late diagnosis, where the 5-year survival rate plummets well below 50%. It is now believed that a significant fraction of ovarian high grade serous carcinoma (HGSC) may actually start in the fallopian tubes (FTs), and that precancerous changes are detectable before metastasis to the ovary and peritoneal cavity occurs. A recently published molecular evolutionary analysis identifies a window of 7 years between development of a serous tubal intraepithelial carcinoma (STIC) and initiation of ovarian carcinoma, with metastases following rapidly thereafter.

The authors are working to address the unmet clinical need for a minimally invasive test for early (pre-metastatic) ovarian cancer. Existing whole-body imaging techniques and transvaginal ultrasound have thus far displayed inadequate resolution and sensitivity, however optical imaging methods could fulfill the criteria for detection of primary HGSC when it is still located in the FTs and before it has spread to the ovaries. We have shown that two optical imaging modalities, optical coherence tomography (OCT) and multispectral fluorescence imaging (MFI) have great promise for detection of ovarian and FT abnormalities. In a small *ex-vivo* study, we found that 5 MFI measurements were sufficient to distinguish between normal, cancerous, and benign FT and ovary tissue with 100% sensitivity and specificity. We used laparoscopic OCT in an *in-vivo* study of 17 women and revealed distinct image features for normal, cancerous, and benign abnormalities of the ovary.

For minimally invasive imaging of the fallopian tubes, a sub-millimeter diameter, flexible, steerable endoscope is necessary in order to navigate through the natural orifice of the ostium in the uterus. We have recently prototyped the smallest (0.8 mm diameter) OCT/MFI imaging endoscope with a forward-viewing imaging channel. The system as tested has approximately 70 degree full field of view, a working distance from 1 mm to infinity, and resolution that varies from 35 µm at 1 mm to 180 µm at 10 mm working distance.

Such an imaging test is minimally invasive however not appropriate for wholesale screening of the general population. Therefore, we propose to utilize it as an adjunct confirmatory test after an initial positive or suspicious blood test. Our preliminary data indicate that there are significant changes in serum protein biomarkers in HGSC cases more than 18 months before diagnosis. We have identified FBG and PF4 as pre-diagnostic biomarkers of HGSC with 18-84 months lead time, and have generated and validated a 3-biomarker panel (FBG, PF4, and CA125) that classifies HGSC with >80% sensitivity/>70% specificity and 18-84 months lead time. We have identified overexpression of several glycolytic enzymes in STIC lesions and in human pre-diagnostic serum samples, indicating potential usefulness of these proteins as screening biomarkers and potential utility of glycolysis as imaging biomarker of STIC lesion.

Our continuing work is focused on refining the serum protein biomarker sensitivity and specificity, creating a second-generation falloposcope for a first-in-women feasibility study, and creating an integrated model that incorporates both sets of data into an early EOC detection method.

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DEVELOPMENT OF A MULTI-PROTEIN CLASSIFIER FOR OVARIAN CANCER DETECTION BY SIMULTANEOUS MEASUREMENT OF 92 SERUM PROTEINS ON PROSEEK MULTIPLEX ONCOLOGY II PLATES

Amy P.N. Skubitz[1,2,3,4], Kristin L.M. Boylan[1,2], Kate Geschwind[1,2], Qing Cao[4], Timothy K. Starr[3,4,5], Melissa A. Geller[3,4], Joseph Celestino[6], Robert C. Bast Jr.[6], Karen H. Lu[6], and Joseph S. Koopmeiners[4,7]

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There are no FDA approved screening tools for detecting ovarian cancer in the general population. The two best known ovarian cancer biomarkers, CA125 and HE4, are neither adequately sensitive nor specific when used alone to screen the general population for early stage disease. By using a combination of protein biomarkers for screening, it may be possible to increase the sensitivity and specificity over CA125 alone. In this study, we used Proseek Multiplex Oncology II plates to simultaneously measure the expression of 92 cancer-related proteins in serum using proximity extension assays. This technology combines the sensitivity of the polymerase chain reaction with the specificity of antibody-based detection methods, allowing multiplex biomarker detection and high throughput quantification. We analyzed one microliter of serum from 61 women with advanced stage high grade serous ovarian cancer and compared the values obtained to 88 age-matched healthy women. Principle component analysis and unsupervised hierarchical clustering separated the ovarian cancer patients from the healthy, with minimal misclassification. Data from the Proseek plates for CA125 levels exhibited a strong correlation with previously measured clinical values for CA125 (correlation coefficient of 0.91). CA125 and HE4 were detected at low levels in samples from healthy women, while higher levels were observed in the ovarian cancer cases. We identified 52 proteins that differed significantly ($p < 0.006$) between ovarian cancer and healthy samples; several of which are novel serum biomarkers for ovarian cancer. In total, 40 proteins had an estimated area under the ROC curve of 0.70 or greater. CA125 alone achieved a sensitivity of 93.4% at a specificity of 98%. However, by adding five proteins to CA125, we increased the assay sensitivity to 98.4%, while holding the specificity fixed at 98%. Our data demonstrate that the Proseek technology can replicate the results established by conventional clinical assays for known biomarkers, identify new candidate biomarkers, and improve the sensitivity and specificity of CA125 alone.

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KEY ACHIEVEMENT OF THE FAST PADE TRANSFORM IN MAGNETIC RESONANCE SPECTROSCOPY FOR EARLY OVARIAN CANCER DIAGNOSTICS

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PURPOSE: An excellent candidate for early ovarian cancer detection would be magnetic resonance spectroscopy (MRS), being non-invasive, ionizing-radiation-free, with the potential to identify metabolic features of cancer. To succeed, encoded MRS time signals must be adequately processed. This has not been feasible in clinical MRS which relies upon the fast Fourier transform (FFT), generating low-quality, poorly-informative spectra, with very few metabolites identified. Our meta-analysis shows that cancerous and benign ovarian lesions are inadequately distinguished via FFT-based MRS. Our advanced signal processor, the fast Pade transform (FPT) has high-resolution capacity and is quantification-equipped. We highlight key achievements of FPT-based MRS for ovarian cancer diagnostics.

EXPLANATION OF THE DATA: In studies on noisy in-vitro type MRS data associated with benign and cancerous ovary, the parametric FPT was benchmarked, precisely reconstructing all physical spectral parameters with extremely high resolution. The parametric FPT was successfully applied to MRS time signals encoded *in-vivo* from serous cystic ovarian tumor. Noise was separated out, to identify and quantify densely-packed, often overlapping genuine spectral components. These include recognized and possible cancer biomarkers: phosphocholine, myoinositol, isoleucine, valine, lactate, threonine, alanine, among some 90 metabolites in a narrow spectral range-of-interest. Nearly all these metabolites remain undetected with Fourier-based in-vivo MRS of the ovary.

The previously unexplored properties of the non-parametric derivative FPT (dFPT) have been benchmarked for detection and quantification of phosphocholine, a key biomarker of ovarian cancer and other malignancies. This is a very demanding task for this shape estimation, since phosphocholine is invisible in customary non-derivative envelopes. The dFPT solves this problem, clearly identifying and quantifying all genuine resonances, including phosphocholine. Thereby, on the same screen, phosphocholine is visualized, as are the concentrations and other peak signatures for all the other metabolites. Thus, the higher-order differentiation transform in the dFPT simultaneously enhances resolution, suppresses noise and exactly quantifies, despite non-parametric processing of envelope lineshapes alone. In sharp contrast, even at low differentiation order, the derivative FFT hugely amplifies noise, losing all genuine information.

With increased derivative order, the non-parametric dFPT exactly reconstructs the components of the parametric dFPT. The peak signatures (positions, heights, widths) reconstructed by the non-parametric dFPT in the magnitude mode are uniquely related to the absorptive non-derivative parametric FPT. This permits straightforward interpretation and extraction of peak area and associated metabolite concentrations. Thus, in the performed benchmarking, the higher-order non-parametric dFPT is a stand-alone method for clear display with identification and exact quantification of key metabolic information, including for the ovarian cancer biomarker phosphocholine.

CONCLUSIONS: Derivative magnetic resonance spectroscopy provides clearly interpretable spectra for clinicians, with all needed quantitative information readily at-hand. Being computationally fast, with robust noise suppression, the dFPT is poised to be implemented in clinical scanners. These proof-of-concept studies with in-vitro type data justify pursuing this strategy in-vivo for ovarian cancer diagnostics. We anticipate that the high-order non-parametric dFPT will be the stand-alone method-of-choice for streamlined detection and exact quantification of key ovarian cancer biomarkers for in-vivo encoded MRS time signals. Work is underway to carry-out these next steps.

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COMPARISON OF POTENTIAL OVARIAN CANCER BIOMARKERS BY MASS SPECTROMETRY-BASED PROTEOMIC ANALYSIS OF RESIDUAL PAP TEST FLUID, CERVICAL SWABS, AND TUMOR TISSUE FROM AN OVARIAN CANCER PATIENT

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Early detection is the key to increased survival for women with ovarian cancer, yet a screening tool has yet to be developed that is adequately sensitive and specific enough for use in the general population. In contrast, screening for cervical cancer by Pap tests has been routinely performed for over 50 years. In the liquid-based Pap test, cells are collected from the cervix and placed into an alcohol-based fixative and then examined for abnormal cells. Since ovarian cancer cells have been observed in Pap tests, we reasoned that ovarian cancer peptide biomarkers may also be present. Our central hypothesis is that proteins shed by ovarian cancer cells can be detected during routine Pap tests by mass spectrometry (MS)-based proteomics. In particular, when collected at the time of cervical cancer Pap test screening, the alcohol-based Pap test fixative and cervical swabs are ideal for biomarker discovery since they are derived from a site near the ovarian cancer (i.e. proteins may be secreted or shed from the tumor and flow through the fallopian tube into the uterus and out the cervical opening). Recently, the fimbria of the fallopian tube have been suggested to be the true precursor to ovarian cancer, strengthening our hypothesis that ovarian cancer proteins will be found in the lower genital tract, perhaps even at early stages. To demonstrate the feasibility of using Pap tests as a biospecimen for proteomics, we previously examined the proteins present in residual Pap test fixative samples from women with normal cervical cytology by MS and described 152 proteins in the "Normal Pap Proteome." The objective of this study was to identify and compare the proteins from three different sources from the same ovarian cancer patient: (i) the residual Pap test fixative, (ii) a Merocel swab of the cervix, and (iii) the primary ovarian cancer tumor tissue. Proteins were concentrated from the cell-free supernatant of the Pap test fixative or eluted from the swab, and then trypsin digested using the filter-aided sample preparation method. A total protein extract from the patient's tumor tissue was digested by standard in-solution trypsin digestion. The samples were run on 2D-liquid chromatography MS/MS, followed by bioinformatics integration. We identified over 5000 proteins total in the three samples. More than 2000 proteins were expressed in all three ovarian cancer samples, including several known ovarian cancer biomarkers such as CA125. By Scaffold analysis of the Gene Ontology nomenclature of the proteins, we classified the proteins by both cellular localization and biological processes. Additional matched samples from patients will be used to build a library of proteins and peptides that are specific to ovarian cancer for use in the development of targeted MS assays. We conclude that quantification of proteins from Pap test fixatives and cervical swabs will prove to be a rich source of biomarkers for ovarian cancer detection.

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TOWARD A HEMATOPOIETIC STEM CELL-BASED PROPHYLACTIC IMMUNO-GENE THERAPY APPROACH FOR OVARIAN CANCER

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Our ultimate goal is to develop a long-lasting, cost-efficient, and technically simple approach that allows for the immuno-prophylaxis of cancer in patients with high-risk for disease recurrence and, ultimately, in patients with cancer-predisposing inherited mutations. Our approach is based on *in vivo* genetic modification of hematopoietic stem cells (HSCs). Because HSCs are self-renewing and give rise to all blood cell lineages, they provide a life-long source of transgene modified myeloid and lymphoid cells that, during tumor development, infiltrate the tumor and support tumor growth. Our *in vivo* HSC transduction approach involves subcutaneous injections of G-CSF/AMD3100 to mobilize HSCs from the bone marrow into the peripheral blood stream and the intravenous injection of an integrating, helper-dependent adenovirus (HD-Ad5/35++) vector system. In preliminary studies we used an integrating HDAd5/35++ vector expressing GFP and mgtm^{P140K} under the ubiquitously active EF1a promoter. We demonstrated in adequate mouse models that HSCs transduced in the periphery home back to the bone marrow where they persist long-term. Transgene expression in peripheral blood cells can be achieved by O^{6}BG/BCNU injection. Short-term exposure of *in vivo* transduced mice to low O^{6}BG/BCNU doses resulted in stable GFP expression in 80% of peripheral blood cells and, in mice with implanted syngeneic tumors, in 80% of tumor infiltrating leukocytes. The predominant GFP-positive cell type in two syngeneic tumor models was tumor-associated neutrophils (TANs) (Ly6G$^+$) and macrophages (TAMs) (F4/80$^+$/MHCII$^+$). GFP$^+$ TANs and TAMs were detectable at very early stage of tumor development. To avoid adverse reactions using our *in vivo* HSC transduction/selection approach, the expression of therapeutic transgenes has to ***i)*** be localized to the tumor, ***ii)*** be automatically activated only when the tumor begins to develop, and ***iii)*** cease when the tumor disappears. To develop such an expression system, we determined (by miRNA-Seq and miRNA-array) the micro-RNA profile in GFP$^+$ cells isolated from the bone marrow HSCs, spleen, PBMCs, and tumors of *in vivo* transduced mice. We found four miRNAs that were expressed at high levels in HSCs, splenocytes and PBMCs, but were absent in tumor-associated leukocytes. By inserting four copies of the corresponding target sites into the 3'UTR of our GFP transgene, the corresponding mRNA is degraded in all cells except tumor-associated leukocytes allowing for tumor-restricted transgene expression. Studies on validating this system using GFP as a reporter gene are nearly completed. Furthermore, we have generated HDAd5/35++ vectors expressing scFv specific to mouse CTLA4 or PDL1 under miRNA control. We have performed *in vivo* HSC transduction-O^{6}BG/BCNU treatment with these vectors and will implant syngeneic tumors at the beginning of August. We expect that intratumoral anti-CTLA4 or anti-PDL1 expression from TANs/TAMs at early stages of tumor development will overcome the immunosuppressive tumor environment allowing effector T-cells to stop tumor growth. The outcome of these studies will be presented. We have also started testing the prophylactic efficacy of our approach in oncogene-transgenic mice that develop spontaneous tumors.

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DESCRIBING THE ODDS OF LONG-TERM SURVIVAL AMONG WOMEN DIAGNOSED WITH HIGH-GRADE SEROUS OVARIAN CANCER USING ELECTRONIC MEDICAL RECORDS

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Relatively little is known about factors associated with long-term survival (LTS) following a diagnosis of ovarian cancer. We conducted a retrospective observational study of high-grade serous ovarian cancer (HGSOC) to explore predictors of LTS, defined as >= 7 years of survivorship, using data from five participating health plans in the Cancer Research Network (CRN, http:\\crn.cancer.gov). Our primary data source was the Virtual Data Warehouse (VDW), a common data model which includes standardized, individual-level data extracted from the electronic data systems of each study site. We included 642 women with incident HGSOC diagnosed between 2000 and 2008 to allow for at least 7 years of follow-up. We conducted a multivariable logistic regression analysis to compare characteristics of women who survived >=7 years after diagnosis (n=148) to those who died within 7 years of diagnosis (n=494). In addition to stage and grade, predictors considered for the model included age, race, smoking status at time of diagnosis, body mass index (BMI) within the year prior to diagnosis, and CA-125 level prior to cancer treatment. We examined estrogen and hormonal contraceptive use, as well as the prevalence of comorbidities in the year prior to diagnosis of ovarian cancer. We also included variables for receipt of surgery, radiation, and chemotherapy. We used forward selection to include variables, and retained them in the final model if they had a p-value of <= 0.3. Our final model included CRN study site, age, stage at diagnosis, CA-125 (categorized as <35 or >=35 units/ml), Charlson comorbidity score, receipt of chemotherapy, BMI, and five separate comorbid conditions: weight loss, hypothyroidism, liver disease, chronic pulmonary disease and hypertension. Of these, only younger age, lower stage, and receipt of chemotherapy were statistically significantly associated with LTS. The OR for LTS was 2.9 (95% CI: 1.2-6.3) for ages 18-49 years at diagnosis compared to >=70 years of age. Those who were diagnosed at stage IV had statistically significantly lower odds of surviving >=7 years than any other stage of disease. The OR for LTS for stage I vs. stage IV was 46.6 (95% CI: 46.7 – 15.9), stage II vs. stage IV was 14.1 (95% CI: 5.3-37.0) and stage III vs. stage IV was 5.3 (95% CI: 2.7-10.4). The OR was 2.9 (95% CI: 1.0-8.4) for receipt of chemotherapy versus no chemotherapy after adjusting for all other covariates. Our analysis of data from electronic medical records did not identify any new characteristics that may be associated with ovarian cancer LTS.

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RNA-SEQUENCING OF FALLOPIAN TUBE – FIMBRIA AND HGSC FROM BRCA MUTATION CARRIERS

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Inheritance of a deleterious mutation in the BRCA1/2 genes increases the risk of the disease by up to 40%. The origin of the disease is still debated; however considerable evidence suggests the fallopian tube as the primary site of disease origin. The fallopian tube epithelium changes morphologically and genomically with the ovulatory cycle. To further understand the transcriptomic profile of the fallopian tube epithelium amongst patients with and without a BRCA mutation who have undergone prophylactic surgery, samples with clinically annotated ovulatory cycle status were analyzed by next-generation sequencing (RNA-Seq). The results from this study will provide an understanding of the origins of the disease and how new therapeutic and preventative interventions can be applied early in the development of the disease.

METHODS: In total a cohort of 68 archival formalin fixed paraffin embedded (FFPE) fallopian tube -fimbria was amassed: FTE-BRCA1 (n=32), FTE-BRCA2 (n=4), chemonaive HGSC-BRCA1 (n=9), FTE-nonBRCA (n=26). The pre-menopausal fallopian tube specimens were sub-divided by ovulatory cycle: normal-Follicular, normal-Luteal, BRCA-Follicular, BRCA-Luteal. The majority of the FTE specimen were obtained from pre-menopausal women (post-menopausal n=2). Laser capture microdissection was performed on the distal end of the fallopian tubes – the fimbria. Six-twelve, 10um FFPE sections were cut and stained with hematoxylin prior to LCM. RNA was extracted using the Roche High Pure FFPE Micro Kit and samples were processed using Illumina Tru-Seq Stranded Total RNA Kit with RiboGold ready and sequenced on the Illumina Hi-seq 2000 V3. Raw results, in FASTQ format, were then processed through the RNA sequencing pipeline to generate results including gene expression data.

RESULTS: Gene expression differences between carriers and non-carriers (FTE-BRCA1/2 and FTE-nonBRCA) revealed genes involved in metabolic pathways namely: oxidative phosphorylation, mitochondrial functions, glycolysis/gluconeogenesis and glycan biosynthesis and metabolism: UGT2A1, ST6GALNNAC6, Complex 1 (ND1, ND2, ND4, NDL4, ND5), ATP6/ATP8, and COX1-3. A comparison between the tumor and fimbria cases showed increased activity in the HMGBI Signaling (increase in HAT1, KAT2B, LIF, JAK3 and PIK3CA and decrease in RAP1A/B, ATM and HMGB1).

CONCLUSION: These results highlight BRCA1/2 distinct unique preneoplastic processes not previously identified. The fallopian tube epithelial in the fimbria in BRCA1 and BRCA2 mutation carriers have increased metabolic activity, indicated by their gene expression profiles. HGSC developed BRCA1 germline mutation carriers have an increased histone acetyltransferase activity shown to be involved in BRCA mediated DNA damage repair. Further analysis is required to understand how the ovulatory cycle influences the fallopian tube epithelium transcriptome, but preliminary results suggest that pathways altered include: metabolic pathways, apoptosis pathways, p53 pathways and mismatch repair pathways. These results support previous findings in addition to providing new insight into the early development of the disease.

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THE EFFECT OF AGE AND OCP USE ON THE INCIDENCE OF PRE-CANCEROUS P53 LESIONS AND THE DEVELOPMENT OF HIGH GRADE SEROUS OVARIAN CARCINOMA

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BACKGROUND: High grade serous ovarian cancer (HGSOC) accounts for >70% of ovarian cancer related deaths and is the most common ovarian cancer histotype, most originating from pre-cancerous p53 lesions in the fallopian tube (FT) fimbria. Use of oral contraceptive pills (OCPs) for 5 years or more is associated with >40% reduction in risk of HGSOC, but the mechanism is unknown. We hypothesize that OCP use reduces the incidence of p53 lesions. Our preliminary data show higher incidence of p53 lesions in post- compared to pre-menopausal women, therefore we aim quantify p53 lesions in post-menopausal women who previously did or did not use OCPs. This will provide insight into the protective effects of OCPs against HGSOC.

PRELIMINARY RESULTS: We determined the presence of p53 lesions by immunohistochemistry (IHC) in FT of women up to 40 years old (n=27) and >60 years old (n=24) who underwent salpingectomies for non-cancer reasons. p53 lesions were identified in 3/27 cases of the younger cohort (11%) and in 10/24 of the older cohort (42%). Thus, we conclude an increased incidence of p53 lesions in older compared to younger women.

PROPOSED DESIGN: IHC for p53 will be performed on FT fimbria of women >55 years old who received salpingectomy/ hysterectomy for non-cancer reasons. Based on an assumed reduction in p53 lesions of 35% in women who used OCPs for 5 years or more compared to non-users (25 vs. 42%), analysis of 190 cases from each group will provide >80% power ($p<0.05$). Cases will be identified through Population Data BC and blind analysis by tp53 IHC will be performed at the Vancouver General Hospital. Post-menopausal status will be confirmed by endometrium histology and data flowed back to Pop Data BC to compare to OCP data.

CONCLUSION: Our preliminary study found that 42% of post menopausal women had p53 lesions, informing this study design. The study registered through this abstract will be the first to examine the impact of OCPs on the earliest known precursors of HGSOC.

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POLYCYSTIC OVARY SYNDROME AND OVARIAN CANCER RISK: A MENDELIAN RANDOMIZATION ANALYSIS

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BACKGROUND: Polycystic ovary syndrome (PCOS), a complex endocrine disorder that has an estimated prevalence of 4-21% in reproductive aged women, is characterized by oligomenorrhea (i.e. infrequent or irregular periods) and abnormal hormone levels including hyperandrogenism, hyperinsulinemia, and gonadotropin imbalance which could influence ovarian cancer risk. Recently, the Ovarian Cancer Association Consortium (OCAC) reported a non-significant decreased risk of invasive ovarian cancer among women with self-reported PCOS. When infrequent and irregular periods were examined as a proxy for PCOS, women reporting these characteristics had a decreased risk of invasive ovarian cancer that was consistent across most histotypes. However, given the limitations of self-reported PCOS, potential confounding, and that oligomenorrhea only captures one facet of PCOS, the causality of these observed associations remains uncertain. Therefore, we sought to use Mendelian randomization (MR), the analytical method that capitalizes on the random assortment of genes from parents to offspring, to examine the association between PCOS and ovarian cancer independent of exposure misclassification and confounding variables.

METHODS: We conducted a literature search to identify single nucleotide polymorphisms (SNPs) associated with PCOS for use as instrumental variables. Using summary statistics from a previously conducted genome wide association study (GWAS) of ovarian cancer among European ancestry women within OCAC (22,406 invasive cases and 40,941 controls), we assessed the association between genetically predicted PCOS and ovarian cancer risk using an inverse-variance weighted method. The associations were examined overall and by histotype. In addition, as oral contraceptives have a well-established protective effect on ovarian cancer risk and are a first-line treatment for women with PCOS to manage menstrual irregularities, hyperandrogenism, and acne, we evaluated the association between PCOS-associated SNPs and oral contraceptive use using publicly available GWAS data.

RESULTS: We identified 7 SNPs that were associated with PCOS on genome-wide significance levels in European populations. A statistically significant inverse association was observed between genetically predicted PCOS and invasive ovarian cancer risk with an odds ratio (OR) of 0.89 (95% confidence interval [CI] = 0.82-0.97; p=0.006). When results were examined by histotype, there was a statistically significant inverse association between genetically predicted PCOS and the high-grade serous (OR=0.88; 95% CI=0.79-0.97; p=0.012; cases=13,307) and endometrioid (OR=0.73; 0.60-0.88; p=0.001; cases=2,810) histotypes. None of our instrument SNPs were associated with ever use of oral contraceptives after adjusting for number of tests. We conducted a sensitivity analyses of our MR analyses excluding two SNPs that showed nominal association (p<0.05) with oral contraceptives and the association with ovarian cancer was not materially changed.

CONCLUSION: Our study provides evidence for a causal relationship between PCOS and ovarian cancer risk, with PCOS reducing risk of most histotypes of ovarian cancer. These results lend support to our previous observational study results. Future studies are needed to understand the mechanisms underlying this association.

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SILICA GEL ENCAPSULATION AS A POTENTIAL TOOL TO IDENTIFY DORMANT AND DRUG-RESISTANT CELL POPULATIONS IN OVARIAN CANCER

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Despite advances in standard of care treatment, a significant number of ovarian cancer patients will exhibit disease recurrence, either due to the presence of chemoresistant cells or the reawakening of dormant tumor cells. Currently there are no methods to predict which patients are likely to recur, and the mechanisms that allow a unique subpopulation of cancer cells to enter dormancy and survive chemotherapy remain unclear. Since chemotherapy is more effective at destroying highly proliferative cells, it is theorized that tumor cells capable of entering a dormant state are also the same population able to evade initial treatment and ultimately engender chemoresistance. A silica gel encapsulation platform that uses physical confinement to inhibit proliferation has previously demonstrated the ability to distinguish between dormancy-capable and dormancy-incapable breast cancer cell lines. In this work, we sought to apply this platform to an ovarian cancer cell line and determine if it could be used to select for the dormancy-capable subset of cells and to detect differences upon enrichment of dormant or chemoresistant cell populations. Upon encapsulation in silica gels, surviving OVCAR-3 cells demonstrated hallmarks of dormancy, including reduction in Ki67 expression, modulation of key cell cycle regulatory genes and dormancy-associated signaling pathways, and ability to resume proliferation when the stress of physical confinement was removed. Moreover, enrichment of the dormant subpopulation using hypoxia treatment or serum starvation prior to silica gel encapsulation resulted in enhanced survival in gels relative to cells grown in standard culture conditions. These results suggest that silica gel encapsulation may be used as a simple method to isolate dormancy-capable cells for further applications such as gene or protein expression analysis and drug screening. Similarly, cells already in a dormant state demonstrated lower susceptibility to cisplatin drug treatment relative to actively proliferating cells, further supporting the idea that dormant cancer cells are less susceptible to chemotherapy and that chemoresistance may stem from a subpopulation of dormant cancer cells. After extraction from silica gels and subsequent return to a proliferative state, cells surviving prolonged encapsulation also demonstrated enhanced survival after cisplatin treatment relative to control populations. This suggests that the dormancy-capable cells selected for within silica gels are indeed more chemoresistant, even when no longer dormant, and that the ability to enter dormancy and chemoresistance in ovarian cancer are likely somehow connected. Lastly, when the drug-resistant phenotype was selected for through cisplatin drug treatment prior to encapsulation, a significant difference in viability was observed upon encapsulation between cisplatin-treated and untreated cells. Overall, these results indicate that the silica gel technology and the behavior of ovarian cancer cells after encapsulation may be an indicator of cells inherently capable of entering dormancy and less susceptible to platinum-based chemotherapies. In future applications, silica gel encapsulation of patient ovarian cancer cells could potentially be used as a predictive clinical tool to identify patients at risk of early recurrence or as a facile in vitro platform to investigate mechanistic links between dormancy, chemoresistance, and recurrence.

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FULL-TERM BIRTHS ARE PROTECTIVE FOR OVARIAN CANCER – WHAT ABOUT INCOMPLETE PREGNANCIES?

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BACKGROUND: Ovarian cancer is associated with reproductive and hormonal factors including parity, breastfeeding, and use of oral contraceptives. However, the effect of incomplete pregnancies, including both induced and spontaneous abortions, is unclear. The most recent assessments of this association include analyses of two Australian case-control studies and the European Prospective Investigation into Cancer and Nutrition (EPIC) study in which both report no significant association between incomplete pregnancies and risk of ovarian cancer. However, a review of these findings and those included in a systematic review are suggestive of an overall protective effect among women who have had an incomplete pregnancy compared to those who have not.

METHODS: To study the incomplete pregnancy-ovarian cancer association in detail, we pooled epidemiologic data from 16 population-based case-control studies from the Ovarian Cancer Association Consortium (OCAC). An incomplete pregnancy was defined as any pregnancy lasting fewer than six months in duration that did not result in a live birth. The effects of ever having an incomplete pregnancy as well as the number of incomplete pregnancies were evaluated using conditional logistic regression after considering potential confounders. Histotype-specific analyses were also conducted to determine the association between incomplete pregnancies and high-grade serous, low-grade serous, mucinous, endometrioid, and clear cell ovarian cancer.

RESULTS: A total of 11,217 ovarian cancer cases and 18,588 controls was included in our analysis. We found that women who ever had an incomplete pregnancy had a 17% decreased risk of ovarian cancer compared to those who had not (95% CI 0.78-0.87). Risk of ovarian cancer also decreased with increasing number of incomplete pregnancies (p-trend<0.001). When the association with ever having an incomplete pregnancy was examined by histotype, the protective association was strongest for clear cell ovarian cancer (OR=0.55, 95% CI 0.46-0.66) and less apparent for high-grade serous and mucinous ovarian cancers (OR=0.94, 95% CI 0.88-1.01 and OR=0.89, 95% CI 0.74-1.07, respectively). The same patterns are observed for full-births.

CONCLUSIONS: Incomplete pregnancies appear to be associated with a decreased risk of ovarian cancer, particularly for the clear cell histotype. Full births are more protective than incomplete pregnancies. However, these findings suggest that the hormonal milieu during pregnancies of short gestation may be sufficient to confer some protection against ovarian carcinogenesis.

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INTRAPERITONEAL CHEMOTHERAPY USE AND OUTCOMES: EXPLORATION OF UPMC OVARIAN CANCER REGISTRY DATA

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BACKGROUND: Ovarian cancer (OC) is the deadliest gynecologic cancer. Despite randomized clinical trials showing improved survival for patients receiving intraperitoneal chemotherapy (IP), IP has not received a wide use outside of specialty hospitals. The aim of this study was to explore the impact of IP on OC patient survival and to evaluate whether treatment facility type impacted the outcomes.

METHODS: Detailed demographic and clinical information on OC patients (N=2924) who underwent treatment in UPMC facilities between 2000-2016 was obtained from the UPMC Cancer Registry. Duplicate records, rare, grade 1, and non-epithelial tumors were excluded. Kaplan-Meier plots were constructed to compare 10-year survival rates based on the chemotherapy type (IP vs. no-IP), cancer grade, cancer stage, surgery type, neoadjuvant and treatment facility (specialized vs. community). Multivariable Gray's models and logistic regression model were fitted to evaluate the effect of different factors on survival and tumor recurrence respectively. Two tailed P-values <0.05 were considered significant. R software package was used to analyze the data.

RESULTS: The final sample consisted from 1840 patients (250 IP and 1590 no-IP). IP chemotherapy was used only in 14% of OC patients and was associated with improved long-term survival. Similarly, cases reported by specialty treatment facilities (Magee and Passavant) had better survival compared to other hospitals. Multivariable Gray's model showed that IP and younger age were significantly associated with lower hazard of death, whereas higher cancer stage is significantly associated with higher hazard of death. Multivariable logistic regression showed that IP is not significantly associated with recurrence after adjusting for median income in the zip code of patient residence and cancer stage.

CONCLUSIONS: These findings demonstrate enhanced long-term survival of patients treated with IP therapy at specialty centers. Increasing IP therapy use in clinical practice for OC patient treatment may be important strategy to improve OC outcomes.

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PRE-DIAGNOSIS USE OF MENOPAUSAL HORMONE THERAPY ASSOCIATED WITH BETTER OVARIAN CANCER SURVIVAL

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BACKGROUND: Several lifestyle factors have consistently been associated with survival of ovarian cancer, including body mass index, analgesic medications, physical activity, and smoking. Menopausal hormone therapy (HT), which can comprise of estrogen only (ET) or a combination of estrogen and progestin (EPT), has also been studied for its impact on ovarian survival. Randomized trials as well as a recent meta-analysis have shown better survival for women who took HT after their diagnosis in comparison to women who did not (HR=0.67, 95% CI 0.47-0.97). However, the relationship between use of HT prior to ovarian cancer diagnosis and survival is less clear.

METHODS: Approximately 4,700 ovarian cancer patients across nine population-based studies participating in the Ovarian Cancer Association Consortium (OCAC) were included in our analysis. Information on HT use prior to diagnosis and potential confounders was obtained via phone or in-person interviews. To estimate the effect of pre-diagnostic HT use on ovarian cancer survival, hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using Cox proportional hazards regression models with left and right truncation to account for different observation times. Effects by type and duration of HT use as well as disease histotype were also evaluated.

RESULTS: Women who used HT before diagnosis had an 11% decreased risk of death in comparison to those who did not use HT (95% CI 0.82-0.97). Similar effects were observed for both types of HT (HR=0.86, 95% CI 0.75-0.98 for ET and HR=0.89, 95% CI 0.80-0.99 for EPT). In addition, there appeared to be a duration effect with longer HT use conferring better survival (p-trend=0.07 for ET, p-trend=0.003 for EPT). When histotype was considered, the overall decreased risk was driven by serous and mucinous ovarian cancers (HR=0.85, 95% CI 0.77-0.93 and HR=0.54, 95% CI 0.28-1.07, respectively).

CONCLUSIONS: Use of HT after diagnosis also affords a survival advantage based on published observational and clinical trials data. We now report that pre-diagnosis HT use may confer a survival advantage among women with ovarian cancer. Interestingly, use of ET prior to diagnosis is associated with increased risk of ovarian cancer, but better survival among women diagnosed with the disease. These observations taken together demonstrate the complex nature of ovarian cancer.

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STUDYING THE SIGNALING PATHWAYS OF DISTINCT SUBPOPULATIONS OF CELLS GENERATED UPON PTEN DELETION FROM FTE AND IDENTIFICATION OF MARKERS OF EARLY TUMORIGENESIS

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SIGNIFICANCE: High Grade Serous Cancer (HGSOC) Requires Novel Biomarkers for early detection.

Survival rates for HGSOC patients have not substantially improved, over the past decades, mainly because the disease is only detected at the metastatic stage. The findings of early pre-malignant lesions in the fallopian tube, but not in the ovary, suggested that HGSOCs may originate from the fallopian tube epithelium (FTE) and metastasize to the ovary. Studying the mechanisms of HGSOC genesis from the correct cell of origin is critical to the early detection of the disease. Our main goal is to determine how genetic and molecular alterations contribute to the early events of HGSOC genesis from FTE. Recent transgenic animal models of FTE-derived HGSOC have pointed out the critical role of loss of PTEN (phosphatase and tensin homolog) from FTE in generating HGSOC when in combination with p53 mutation and alteration of BRCA1/2, but not by itself. Our lab was the first to show that deletion of PTEN alone in FTE is sufficient to lead to tumor formation and peritoneal dissemination suggesting that the importance of PTEN in the early events of the tumorigenic cascade may have been underestimated.

Loss of PTEN from FTE is sufficient to drive ovarian cancer, however, the key signaling driving tumorigenesis when PTEN expression is low remain poorly defined and yet pivotal to new treatment options given its common occurrence. Studying the progression of HGSOC genesis from FTE is essential to refining the strategy for targeted therapies and to discovering novel biomarkers for early detection of the disease.

PURPOSE*:* *to identify signaling pathways and drug response of different subpopulations of tumorigenic cells generated upon PTEN deletion.*

RESULTS: Loss of PTEN in the FTE upregulates markers of cancer stem cells (CSC) such as WNT4, LGR5, ALDH1, c-Kit, CD44, and Dll4. We also discovered that the CSC markers are confined in a specific subpopulation of cells with increased diameter that we call CSC^{High} as compared to a smaller population that we call CSC^{Low}. In fact, when these two populations are separated by flow cytometry based sorting, the CSC^{High} retains all the CSC markers. In addition, using a limiting dilution assay, we found that the CSC^{High} subpopulation forms tumor at a faster rate ***in vivo***. In addition, CSC^{High} forms bigger spheroids and are more resistant to chemotherapeutic agents, suggesting that more targeted therapy may be developed for these stem-like, tumorigenic subpopulations. We also show that loss of PAX2 is mediating the CSC^{High} phenotype suggesting that therapies to re-express PAX2 may prevent development of HGSOC.

CONCLUSIONS: Together, these findings provide a novel model to study the mechanism of fallopian tube tumor initiation and invasion to the ovary mediated by loss of PTEN, which may help to define early events of human ovarian carcinogenesis.

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DEVELOPMENT AND VALIDATION OF CIRCULATING CA125 PREDICTION MODEL IN POSTMENOPAUSAL WOMEN WITHOUT OVARIAN CANCER

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BACKGROUND: Cancer antigen 125 (CA125) is a membrane bound glycosylated mucin which has been reported to be the most promising biomarker for ovarian cancer screening. However, results from two large randomized trials comparing screening with CA125 and transvaginal ultrasound to usual care have shown no clinically significant difference in ovarian cancer mortality. A major limitation of CA125 as an ovarian cancer screening biomarker has been low specificity and variation between individuals by personal characteristics. Identifying personal characteristics that influence CA125 levels could be used to create personalized thresholds for CA125 thereby improving its performance as an ovarian cancer screening biomarker. We developed and conducted internal and external validation of two prediction models (linear and dichotomous) of circulating CA125 among postmenopausal women using 28,842 controls without ovarian cancer in four large population-based studies.

METHODS: We identified controls from three prospective cohort studies, including Prostate, Lung, Colorectal, and Ovarian (PLCO, n=26,981), European Prospective Investigation into Cancer and Nutrition (EPIC, n=861), and the Nurses' Health Studies (NHS, n=164) as well as one population-based case-control study, the New England Case Control Study (NEC, n=1,000). CA125 was measured using the CA125II assay in PLCO, NHS, and NEC. Meso Scale Discovery (MSD) platform was used to measure CA125 in EPIC. The MSD assay values were recalibrated to the CA125II scale based on 534 NEC controls with both measurements. CA125 levels were log-transformed to achieve normal distribution or dichotomized by the upper limit of normal (35 U/ml). The prediction models were developed and internally validated using postmenopausal controls in PLCO, and then were externally validated using postmenopausal controls in EPIC, NHS and NEC. The prediction models were developed using stepwise linear or logistic regression with <0.15 as significance level for entry and retention considering factors which have been previously reported to be associated CA125 in postmenopausal women as candidate predictors (age, race, body-mass index (BMI), smoking status and duration, age at menarche, oral contraceptive use, party, age at menopause, time since menopause, hormone replacement therapy (HRT) use and duration, family history of ovarian or breast cancer, previous history of cancer, previous history of benign ovarian cyst, history of endometriosis). We then evaluated the performance of the model in the independent validation datasets.

RESULTS: The linear CA125 prediction model included age, race, BMI, smoking status and duration, hysterectomy, parity, age at menopause, and duration of HRT use as predictors, explaining 5% of the variability of log-transformed CA125 levels. The correlation coefficient of the measured and predicted log-transformed CA125 was 0.18 in the PLCO testing dataset, and showed comparable correlations across the independent validation datasets (0.14-0.16). The dichotomous CA125 prediction model included age, race, BMI, duration of HRT use, and hysterectomy as predictors with an AUC of 0.63 in the PLCO testing dataset and 0.71 in NEC.

CONCLUSION: We developed linear and dichotomous circulating CA125 prediction models in postmenopausal women that can form the foundation for creating personalized thresholds of CA125. However, other factors should be considered to increase the predictive capacity of the model.

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LIFETIME NUMBER OF OVULATORY CYCLES ARE DIFFERENTIALLY ASSOCIATED WITH OVARIAN CANCER HISTOTYPES: AN ANALYSIS FROM THE OVARIAN CANCER COHORT CONSORTIUM (OC3)

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BACKGROUND: Epidemiologic studies have consistently observed reduced ovarian cancer risks with higher parity and oral contraceptive use. Increased risks with younger age at menarche and older age at menopause have also been reported. Furthermore, it has been demonstrated that an acute pro-inflammatory environment is created following ovulation at the surface of the ovary and within the distal fallopian tube, whereby both are bathed in follicular fluid containing inflammatory cytokines, reactive oxygen species, and steroids, creating a DNA damage-rich environment and thereby supporting the possible role of incessant ovulation in ovarian carcinogenesis. Consistent with this, greater lifetime ovulatory cycles (LOC) have been associated with increased ovarian cancer risk in numerous studies, however the etiologic heterogeneity of this association has not been resolved. It is difficult to measure LOCs directly, but estimates of the cumulative sum of a woman's ovulatory cycles can be obtained through mathematical algorithms that calculate the time between menopause and menarche (menstrual span) and subtract anovulatory cycles, i.e., durations of oral contraceptive use and pregnancy.

AIM: To investigate the association of LOC and its components with ovarian cancer (overall and by histotype) using prospective individual-level data from the Ovarian Cancer Cohort Consortium (OC3).

METHODS: We analyzed data from 23 prospective cohort studies including 3,866 ovarian cancer cases diagnosed among 618,175 naturally menopausal women. Cases included 2288 serous, 352 endometrioid, 210 mucinous, and 137 clear cell tumors, and 879 other epithelial/unknown tumors. We evaluated associations between LOC, individual components of LOC (menstrual span, pregnancy, oral contraceptive use), and ovarian cancer using Cox regression stratified by study and adjusted for potential confounders; histotype analyses were conducted using competing-risks Cox regression.

RESULTS: In models evaluating the overall LOC effect (without adjustment for component factors), women in the 90th percentile of LOC (>511) were almost twice as likely to be diagnosed with ovarian cancer during follow-up than women in the 10th percentile (<295 cycles) [hazard ratio (HR) (95% confidence interval (CI): 1.92 (1.62-2.62)]. Per one-year increase in LOC (12 cycles), ovarian cancer risk was increased by 2.5% [1.025 (1.02-1.03)]. This association was heterogenous by histotype; a one-year increase in LOC was associated with increased risk of serous [1.026 (1.02-1.03)], endometrioid [1.04 (1.02-1.06)], and clear cell tumors [1.07 (1.04-1.10)], while no association was observed for mucinous tumors [1.00 (0.98-1.02), p-heterogeneity<0.01]. Adjusting for LOC-components, pregnancy and oral contraceptive use, the LOC-ovarian cancer association remained but was attenuated [per year LOC: 1.014 (1.007-1.02)]. The associations were more similar across histotypes in adjusted models (1.02 serous, 1.03 endometrioid, 1.05 clear cell; p-values<0.05), likely due to accounting for stronger risk reductions associated with prior pregnancy among endometrioid and clear cell tumors than serous tumors.

CONCLUSIONS: In this large prospective analysis of pooled cohort study data we observed positive associations between increased LOC and risk of serous, endometrioid, and clear cell tumors, independent of the associations with individual LOC components. Our data provide support for the hypothesis that incessant ovulation contributes to the etiology of these ovarian cancer histotypes, and further supports the etiologic heterogeneity of ovarian cancers.

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BIOTINYLATED ESTROGENS A NOVEL TOOL FOR EARLY DETECTION OF ADDUCT IN OVARIAN CANCER

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Elevated estrogens exert their carcinogenic effects by at least three different mechanisms; they promote cell proliferation by transcriptional regulation of estrogen responsive genes, cause generation of reactive oxygen species and other free radicals, and directly react with DNA and form potentially mutagenic adducts. However, unbalanced estrogen metabolism has been linked to development of several malignant diseases including breast, endometrial and ovarian cancer and has been identified as a risk factor for these cancers. The metabolic breakdown of estogens is regulated by a series of reactions mediated by hepatic and peripheral enzymes that balances the induction and reduction of cellular stress. An imbalance in this enzymes and processes can lead to highly reactive catechol estrogen metabolites that can react with DNA, form carcinogenic DAN-adducts and lesions. Although several studies established a connection between estrogen-induced DNA damage and carcinogenesis, the underlying molecular mechanisms have been difficult to study because of the technical challenges in detecting and analyzing the variety of different DNA lesions that are formed by estrogen compounds. Moreover, detection and analysis of these adducts are important to directly monitor estrogen metabolites induced cellular responses in the cells. Towards this, we developed a novel method using biotinylated-estrogens that allows immunodetection of estrogen-induced DNA adducts by Slot-blot and single-cell molecular combing and proximity ligation assays. Using these modified estrogens we first time quantitatively detected these adducts on DNA by immune Slot-blot techniques and on DNA fibers. Furthermore, similar to other environmental carcinogens estrogens activates replication associated DNA damage responses and induces chromosomal instability. Hence, first time our studies demonstrate that biotin-labeled estrogens could be a powerful tool to detect estrogen adducts and to probe associated DNA damage responses and cellular responses.

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SESSION 2: GENOMICS AND MOLECULAR MECHANISMS OF OVARIAN CANCER

INVITED SPEAKERS

ABSTRACT PRESENTATIONS

SESSION 2: GENOMICS AND MOLECULAR MECHANISMS OF OVARIAN CANCER (POSTER PRESENTATIONS)

80 **POSTER GMM–016 | SESSION II: FRIDAY**
Cerebellin-2 (CBLN2) is differentially expressed in ovarian cancer subtypes
John D. Andersen, MD
Womack Army Medical Center

81 **POSTER GMM–017 | SESSION I: THURSDAY**
Upregulation of miR-328 contributes to ovarian cancer stem cell maintenance by downregulating DDB2
Ananya Banerjee, MSc
The Ohio State University

82 **POSTER GMM–018 | SESSION II: FRIDAY**
CD73: a new driver and a therapeutic target in ovarian cancer stem cells
Ugo Cavallaro, PhD
European Institute of Oncology (Italy)

83 **POSTER GMM–019 | SESSION I: THURSDAY**
MEK inhibitor trametinib in high grade ovarian cancer proliferation
Ilana Chefetz, PhD
University of Minnesota

84 **POSTER GMM–020 | SESSION II: FRIDAY**
Cell of origin, mutation and microenvironment: Modeling early events of endometriosis associated cancers
Dawn R Cochrane, PhD
BC Cancer Agency (Canada)

85 **POSTER GMM–021 | SESSION I: THURSDAY**
Utilization of patient-derived tumor xenograft (PDX) models in oncology
Magdalena Cybula, PhD
Oklahoma Medical Research Foundation

86 **POSTER GMM–022 | SESSION II: FRIDAY**
IDH1 is a pro-senescent therapy in high-grade serous ovarian cancer
Erika S Dahl, PhD
Penn State College of Medicine

87 **POSTER GMM–023 | SESSION I: THURSDAY**
Patient derived Xenografts (PDXs) and PDX Derived Tumor Cells (PDTC) allow the identification of actionable cancer genes and treatment options for platinum refractory/resistant ovarian carcinomas
Maria Flavia Di Renzo, MD
Candiolo Cancer Institute FPO-IRCCS (Italy)

88 **POSTER GMM–024 | SESSION II: FRIDAY**
Targeting the formin-assembled actin cytoskeleton as an anti-invasion strategy in ovarian cancer spheroids
Kathryn M. Eisenmann, PhD
University of Toledo

89 **POSTER GMM–025 | SESSION I: THURSDAY**
ERBB Family Genomic Alterations in Gynecological Malignancies
Laurie M Gay, PhD
Foundation Medicine, Inc.

90 **POSTER GMM–026 | SESSION II: FRIDAY**
Amplification of USP13 drives ovarian cancer metabolism
Cecil Han, PhD
Georgetown University School of Medicine

91 **POSTER GMM–027 | SESSION I: THURSDAY**
DYNLL1 inhibits DNA end resection in BRCA1-deficient cells and regulates PARP inhibitor sensitivity
Yizhou Joseph He, PhD
Dana Farber Cancer institution

92 **POSTER GMM–028 | SESSION II: FRIDAY**
Validation of RUNX3 and CaMKIIN hypermethylation as prognostic marker for ovarian carcinoma
Karolin Heinze, MSc
Jena University Hospital (Germany)

93 **POSTER GMM–029 | SESSION I: THURSDAY**
Mitochondrial Stress Response Adaptations are Required for Ovarian Cancer Anchorage-Independent Survival and Metastasis
Nadine Hempel, PhD
Penn State College of Medicine

94 **POSTER GMM–030 | SESSION II: FRIDAY**
Models and Analytic Techniques of Mullerian Tissue-Derived Organoids
Germain C. Ho, BSc
BC Cancer (Canada)

95 **POSTER GMM–031 | SESSION I: THURSDAY**
Clinical pathologic expression of cell cycle regulatory complexes in high grade serous ovarian carcinoma
Audra N. Iness, BS
Virginia Commonwealth University

96 **POSTER GMM–032 | SESSION II: FRIDAY**
Unravelling the steroid metabolome in epithelial ovarian cancer
David N. Jeevan, MD
University of Birmingham

97 **POSTER GMM–033 | SESSION I: THURSDAY**
Conditional BRCA2 Switch in Human Cells to Study Tumor Progression
Judit Jimenez–Sainz, PhD
Yale Medical School

98 **POSTER GMM–034 | SESSION II: FRIDAY**
Co-regulation and functional cooperativity between FOXM1 and RHNO1 bidirectional genes in high-grade serous ovarian cancer
Adam R. Karpf, PhD
University of Nebraska Medical Center

99 **POSTER GMM–035 | SESSION I: THURSDAY**
Targeting SPSB1 sensitizes platinum-resistant ovarian cancer cells to cisplatin
Mi-Kyung Kim, MD
Ewha Womans University (Republic of Korea)

100 **POSTER GMM–036 | SESSION II: FRIDAY**
Comprehensive genomic, proteomic, and experimental characterization of ovarian clear cell carcinoma cell lines for improved drug development
Yasuto Kinose, MD, PhD
University of Pennsylvania Perelman School of Medicine

101 **POSTER GMM–037 | SESSION I: THURSDAY**
Carbonic Anhydrase 9 and tumor hypoxia are associated with recurrence and resistance in BRCA1-expressing HGSOC
Adam J. Krieg, PhD
Oregon Health & Science University

102 **POSTER GMM–038 | SESSION II: FRIDAY**
Deciphering the role of Estrogen Receptor Alpha in Ovarian Cancer
Irene I Lee, PhD
Dana Farber Cancer Institute

103 **POSTER GMM–039 | SESSION I: THURSDAY**
Building an organoid-based model for ovarian cancer
Kadi Löhmussaar, MSc
Hubrecht Institute (Netherlands)

104 **POSTER GMM–040 | SESSION II: FRIDAY**
Targeting Wnt Signaling to Overcome PARP Inhibitor Resistance
Alexandra McMellen, BSE, pre-doctoral candidate
University of Denver, Anschutz Medical Campus

105 **POSTER GMM–041 | SESSION I: THURSDAY**
Ovarian cancer cells induce a feedback response to platinum-based chemotherapy by activating an oncogenic RSK-EphA2-GPRC5A signaling switch
Lidia Moyano–Galceran, MSc
Karolinska Institutet (Sweden)

106 **POSTER GMM–042 | SESSION II: FRIDAY**
Pan-Cancer Transcription Factor Analyses Identify ZNF217 as a Novel Driver in HGSOC
Robbin Nameki, BS
Cedars-Sinai Medical Center

107 **POSTER GMM–043 | SESSION I: THURSDAY**
ctDNA profiling to predict prognosis and optimize treatment in high-grade serous ovarian cancer
Jaana Oikkonen, PhD
University of Helsinki (Finland)

108 POSTER GMM–044 | SESSION II: FRIDAY
Identification of a novel candidate biomarker for diagnosis and therapeutic ovarian cancer target
Kyoungsook Park, MD, PhD
Sungkyunkwan University (Republic of Korea)

109 POSTER GMM–045 | SESSION I: THURSDAY
PAX8 plays an essential role in high grade serous ovarian cancer via activation of mutant p53 and mislocalized p21
Ruth Perets, MD, PhD
Rambam Health Care Campus (Israel)

110 POSTER GMM–046 | SESSION II: FRIDAY
CCNE1 and BRD4 expression and platinum resistance in high-grade serous ovarian cancers
Shariska Petersen, MD
University of Kansas Cancer Center

111 POSTER GMM–047 | SESSION I: THURSDAY
Genomic characterization of adult-type granulosa cell tumors: Implications for pathogenesis and treatment of recurrent disease
Jessica A. Pilsworth, PhD
University of British Columbia (Canada)

112 POSTER GMM–048 | SESSION II: FRIDAY
ctDNA response to the PARP inhibitor rucaparib predicts progression-free survival and best overall response on the ARIEL2 trial
Anna Piskorz, PhD
University of Cambridge

113 POSTER GMM–049 | SESSION II: FRIDAY
STAT3 promotes ovarian cancer growth and drug resistance by modulating the energy metabolism
Ramandeep Rattan, PhD
Henry Ford Hospital

114 POSTER GMM–050 | SESSION I: THURSDAY
Identification of Novel Chromosomal Rearrangements in High-Grade Serous Ovarian Cancer
Nanna Sarvilinna, PhD
Biomedicum Helsinki 1 (Finland)

115 POSTER GMM–051 | SESSION II: FRIDAY
A CLIA-certified high-throughput drug screening platform for ovarian cancer to inform personalized cancer care and discover novel combinations
Franz X Schaub, PhD
SEngine Precision Medicine

116 POSTER GMM–052 | SESSION I: THURSDAY
Evidence for APOBEC Mutagenesis in Clear Cell Ovarian Carcinoma
Artur S. Serebrenik, PhD
University of Minnesota

117 POSTER GMM–053 | SESSION II: FRIDAY
Role of LATS and DYRK1A kinases in the pathogenesis and treatment of HGSOC
Fatmata Sesay, PhD
Virginia Commonwealth University

118 POSTER GMM–054 | SESSION I: THURSDAY
An investigation of the effects of the atypical PKC-ζ inhibitor ζ-Stat on clear cell carcinoma ovarian cancer proliferation and invasion
Tracess Smalley, Doctoral Candidate
The University of South Florida

119 POSTER GMM–055 | SESSION II: FRIDAY
BMI1 and RING1A are involved in H2A ubiquitination at sites of platinum-induced damage
Shruthi Sriramkumar, PhD
Indiana University Bloomington

120 POSTER GMM–056 | SESSION I: THURSDAY
IGF-axis signaling during ovulation is responsible for the stemness cell expansion and transformation of fallopian tube fimbrial epithelium
Tang-Yuan Chu, MD
Buddhist Tzu Chi General Hospital (Taiwan)

121 POSTER GMM–057 | SESSION II: FRIDAY
A functional homologous recombination assay predicts primary chemotherapy response and long-term survival in ovarian cancer patients
Manuela Tumiati, PhD
University of Helsinki (Finland)

122 POSTER GMM–058 | SESSION I: THURSDAY
CBX2 identified as driver of anoikis escape and dissemination in high grade serous ovarian cancer
Lindsay J. Wheeler, MD
University of Colorado

123 POSTER GMM–059 | SESSION II: FRIDAY
Shear stress downregulation of miR-199a-3p drives chemoresistance in ovarian cancer cells
Alice S. T. Wong, PhD
University of Hong Kong (Hong Kong)

124 POSTER GMM–060 | SESSION I: THURSDAY
A novel treatment approach for targeting Cyclin E over-expressing ovarian cancers
Haineng Xu, PhD
University of Pennsylvania

125 POSTER GMM–061 | SESSION II: FRIDAY
Molecular Characterization of Mouse Models of High-Grade Serous Carcinoma Arising in the Oviduct
Yali Zhai, MD, PhD
University of Michigan Medical School

126 POSTER GMM–062 | SESSION I: THURSDAY
Targeting ovarian cancer stem cells through the tumor suppressor DAB2IP-mediated Wnt signaling pathway
Xingyue Zong, BS
Indiana University

DNA REPAIR IN OVARIAN CANCER

Alan D'Andrea, MD

Dana Farber Cancer Institute

No abstract provided.

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MODELING THE GENOMICS OF HIGH-GRADE SEROUS CARCINOMA IN THE MOUSE

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No abstract provided.

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COPY-NUMBER SIGNATURES AND MUTATIONAL PROCESSES IN HIGH GRADE SEROUS OVARIAN CARCINOMA

Geoff Macintyre[1,†], Teodora E. Goranova[1,†], Dilrini De Silva[1], Darren Ennis[2], Anna M. Piskorz[1], Matthew Eldridge[1], Daoud Sie[3], Liz-Anne Lewsley[4], Aishah Hanif[4], Cheryl Wilson[4], Suzanne Dowson[2], Rosalind M. Glasspool[5], Michelle Lockley[6,7], Elly Brockbank[8], Ana Montes[9], Axel Walther10, Sudha Sundar[11], Richard Edmondson[12,13], Geoff D. Hall[14], Andrew Clamp[15], Charlie Gourley[16], Marcia Hall[17], Christina Fotopoulou[18], Hani Gabra[18,19], James Paul[4], Anna Supernat[1], David Millan[20], Aoisha Hoyle[20], Gareth Bryson[20], Craig Nourse[2], Laura Mincarelli[2], Luis Navarro Sanchez[2], Bauke Ylstra[3], Mercedes Jimenez-Linan[21], Luiza Moore[21], Oliver Hofmann[2,22], Florian Markowetz[1,*], Iain A. McNeish[2,5,18,*], **James D. Brenton**[1,21,23,*,#]

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BACKGROUND: The genomic complexity of profound copy-number aberration has prevented effective molecular stratification of high grade serous ovarian carcinoma (HGSOC). Recent algorithmic advances have enabled interpretation of complex genomic changes by identifying mutational signatures—genomic patterns that are the imprint of mutagenic processes accumulated over the lifetime of a cancer cell. We hypothesized that specific features of copy-number (CN) abnormalities could represent the imprints of distinct mutational processes, and developed methods to identify signatures from copy-number features in HGSOC.

METHODS: We derived copy-number signatures from absolute copy number profiles from 253 primary and relapsed HGSOC samples from 132 patients in the BriTROC-1 cohort using low-cost shallow whole-genome sequencing (sWGS; 0.1×). A subset of 56 of these cases had deep whole-genome sequencing (dWGS) performed for mutation analysis and comparison with sWGS data. Independent validation was performed using 112 dWGS HGSOC cases from PCAWG and 415 HGSOC cases with SNP array and whole exome sequence from TCGA. CN signature exposures were correlated with mutation data, SNV signatures, and other measures derived from deep WGS and exome sequencing to identify statistically significant genomic associations using a false discovery rate <0.05.

RESULTS: We identified 7 CN signatures that provided a molecular framework to rederive the major defining elements of HGSOC genomes, including defective homologous recombination (HRD), tandem duplication, amplification of *CCNE1* and amplification-associated fold-back inversions. Almost all patients with HGSOC demonstrated a mixture of signatures indicative of combinations of mutational processes, including those with early driver events such as *BRCA2* mutation (in addition to HRD signatures). High exposure to CN signature 3, characterised by BRCA1/2-related HRD, was associated with improved overall survival. Conversely, high exposure to signature 1, which was characterised by oncogenic RAS signaling (including *NF1, KRAS* and *NRAS* mutation), predicted platinum-resistant relapse and poor survival.

CONCLUSIONS: HGSOC lacks clinically-relevant patient stratification, which is reflected in poor survival and is a significant barrier to precision medicine. Copy-number signature exposures at diagnosis predict both overall survival and the probability of platinum-resistant relapse. Our results suggest that early *TP53* mutation, the ubiquitous initiating event in HGSOC, may permit multiple mutational processes to co-evolve, potentially simultaneously and that additional signature exposures may alter the risk of developing therapeutic resistance. Thus, our results suggest that HGSOC is a continuum of genomes.

We derived signatures using inexpensive sWGS of DNA from core biopsies. These approaches are rapid and cost effective, thus providing a clear path to clinical implementation. By dissecting the mutational forces shaping HGSOC genomes, our study paves the way to understanding extreme genomic complexity, as well as revealing the evolution of tumors as they relapse and acquire resistance to therapy.

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REAL-TIME ASSESSMENT OF HGSC DNA DAMAGE REPAIR DEFECTS AND DEFECT-INDUCED RESPONSE TO THERAPY IN OVARIAN CANCER ORGANOIDS

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Patients with High Grade Serous Ovarian Cancer (HGSC) have limited therapeutic options. Immuno-oncologic (IO) agents have had limited effect. DNA damage repair gene mutations that may confer repair defects have been identified in up to 50% of HGSCs, making therapies that target repair defects, like PARP, CHK1, and ATR inhibitors, additional options. We have no means of predicting which patients will respond to any of these therapies.

A model system that allows for functional assays to assess for DNA damage repair defects, prediction of response to therapies targeting such defects, and assessment of the functionality of the tumor immune infiltrate and its response to IO agents is needed. Organoids are three-dimensional structures derived from human normal or tumor tissue cells that anatomically and functionally mimic the developed human organ. Organoids mimicking the parent tumor from which they were derived have aided in the study of multiple tumor types. They are inexpensive and easily manipulated and may be an ideal model system for studying ovarian cancer.

We have devised a functional assay platform to profile the DNA damage repair capacity and immune targetability of short-term patient-derived HGSC organoids. The organoids mimic the tumors from which they were derived morphologically, molecularly, and genetically.

We have tested 33 organoid cultures derived from 21 HGSC patients for homologous recombination (HR) and replication fork protection capacity and compared the functional results to the tumor genomic profile. Regardless of repair gene mutational status, an HR functional defect in the organoids correlated with PARP inhibitor sensitivity. A fork protection functional defect correlated with carboplatin, and ATR and CHK1 inhibitor sensitivity. Importantly, this work has led to the discovery of potential therapeutic combinations, such as a CHK1 inhibitor plus carboplatin or gemcitabine that may be useful in treating tumors otherwise resistant to most therapies. Drugs such as carboplatin or gemcitabine can synergize with a CHK1 inhibitor by enhancing replication stress and fork deprotection.

In parallel, we have immune phenotyped the parent tumors and organoid cultures from 15 patients, and shown that the organoid cultures retain lymphocytes expressing relevant IO receptors in the short term. Upon treatment with carboplatin, olaparib, and pembrolizumab as single agents or in combination, we detect changes in IO receptor expression and production of different cytokines in the cultures, suggesting an immune response induced by these agents. We have detected receptor and cytokine alterations that would create an immune suppressive environment with specific drug combinations in tumors with specific repair defects, suggesting that these may be inappropriate combinations for harnessing the immune system in tumors with specific repair capacities.

Continued combined immune and DNA damage repair phenotyping analyses of the organoids will lead to a better understanding of which mechanistic defects are needed to confer sensitivity to DNA damage repair agents, what functional properties and immune milieu lead to sensitivity to IO agents, and how best to combine such therapies. In addition, through further correlation with patient responses over time, HGSC organoids may become a useful tool for rapidly predicting patient response to therapeutic agents.

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IDENTIFYING FUNCTIONAL NONCODING SOMATIC MUTATIONS IN OVARIAN CANCER

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Epithelial ovarian cancer (OC) is a heterogeneous disease that stratifies into different histologic subtypes including high-grade serous (HGSOC), clear cell (CCOC), endometrioid (EnOC) and mucinous (MOC) ovarian cancer. HGSOC is the most common, and each histotype is characterized by largely distinct germline genetics, somatic alterations and clinical biomarkers. Recently, whole genome sequencing (WGS) studies have catalogued genome-wide somatic variation for most OC histotypes. These data demonstrate that OC histotypes harbor thousands of noncoding somatic mutations and our next major challenge is to distinguish the few important noncoding somatic drivers from the thousands of passenger mutations.

We hypothesized that driver noncoding somatic mutations impact disease development and progression through altering the sequence of regulatory elements (REs), such as enhancers and promoters, resulting in perturbed expression of genes involved in neoplastic development of OC. To systematically address this hypothesis, we established genome-wide H3K27ac epigenomic profiles, annotating active REs for the different ovarian cancer histotypes using chromatin immunoprecipitation sequencing (ChIP-seq) in 20 fresh frozen primary OC tissue samples—five tumors for each major histotype. In parallel, we performed transcriptional profiling using RNA sequencing (RNA-seq). Together, these two datasets enabled us to evaluate epigenetic alterations and the transcriptome.

We identified histotype-specific active REs, and common active REs across all histotypes. First, we used the RNA-seq data to assess the effect of the histotype-specific REs in gene expression, and to find target genes of cis-REs and novel histotype-specific biomarkers. Next, we integrated these unique profiles with WGS data from 232 OCs (169 HGSOCs, 35 CCOCs and 28 EnOCs). The number of somatic single nucleotide variants per sample range from 481 to 40,764 (mean=7199, sd = 5751). Of these, 9.4% were noncoding and overlapped active REs in OC. Using a Poisson binomial distribution, we tested the significance of the observed number of mutated samples for any given active RE. We identified several frequently mutated REs in HGSOC, CCOC and EnOC, including promoters (POLR3E and CHCHD6 for HGSOC, CA5BP1 and C16orf87 for EnOC, DKC1 and SLIT3 for CCOC+EnOC), enhancers and super enhancers (KLF6 for HGSOC). We developed a novel approach to identifying target genes associated with a collection of frequently mutated enhancers by correlating enhancer activity (H3K27ac ChIP-Seq signal) vs. gene expression (RNA-seq signal), identifying HOXD cluster genes and C19orf44 as targets of mutated REs in OC.

In conclusion, we have used a multi-omics method to identify functional, driver noncoding somatic mutations for ovarian cancer based on their interaction with disease-specific regulatory elements. This represents a powerful way to distinguish important noncoding somatic drivers from a much larger number of passenger mutations that accumulate during tumor development.

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FOXM1 INDUCES DNA REPLICATION STRESS AND PROMOTES GENOMIC INSTABILITY DOWNSTREAM OF CYCLIN E1 IN HIGH-GRADE SEROUS OVARIAN CANCER

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PURPOSE: Genomic instability (GI) is a hallmark of high-grade serous ovarian cancer (HGSC), and induction of the FOXM1 transcription factor regulatory network is the second most frequent genetic alteration in HGSC. FOXM1 expression is associated with cancer GI, but the mechanistic link is unknown. DNA replication stress (DRS), which involves altered replication fork dynamics, is a key contributor to cancer GI. FOXM1 and cyclin E1 (CCNE1) are both expressed in STIC lesions in the fallopian tube epithelium (FTE), and CCNE1-CDK2 enhances FOXM1 transcriptional activity. Based on these observations, we hypothesized that: 1) FOXM1 is linked to cancer GI through the induction of DRS, and 2) FOXM1 promotes GI downstream of CCNE1, by promoting unscheduled M phase entry in cells that have not completed DNA synthesis.

METHODS: We determined associations between FOXM1 and markers of DRS and GI in TCGA HGSC data. We overexpressed FOXM1c and a DNA binding domain mutant (DBD) in immortalized FTE cells and characterized the impact on DRS, DNA replication, and DNA damage. We determined the impact of CCNE1 on FOXM1 expression in immortalized FTE cells. We determined the combined effect of CCNE1 and FOXM1 on GI in FTE cells.

RESULTS: FOXM1 was significantly associated with markers of DRS (CHK1-Ser345) and GI (fractional copy number altered genome (CNA)) in TCGA HGSC data. FOXM1 expression, but not its DBD mutant, induced biomarkers of DRS and DNA damage in hTERT/mutant Tp53 immortalized FTE cells. Unexpectedly, FOXM1c increased DNA replication fork rate in FTE cells. Increased fork rate is a novel and recently recognized cause of DRS. CCNE1 expression in FTE cells induced and activated FOXM1, promoting mitotic entry. FOXM1 and CCNE1 co-expression in FTE cells induced an expression signature of GI, and TCGA HGSC tumors with FOXM1 and CCNE1 co-expression showed significantly increased GI.

CONCLUSIONS: We demonstrate that FOXM1 induces DRS and DNA damage in FTE cells, providing an explanation for the known link between FOXM1 and GI. Notably, FOXM1 induces a novel form of DRS involving increased replication fork rate. We show that CCNE1 activates FOXM1, which in turn promotes mitotic entry and GI. These data reveal a potentially major form of oncogenic collaboration in HGSC, and has implications for HGSC treatment approaches that promote DRS or inhibit the cellular DRS response.

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NOTES

GENOME-SCALE CRISPR KNOCKOUT SCREEN IDENTIFIES TIGAR AS A MODIFIER OF PARP INHIBITOR SENSITIVITY

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A key challenge is to understand the mechanisms that contribute to “BRCAness” and hence sensitivity to poly (ADP-ribose) polymerase (PARP) inhibitors. Using the sensitivity to PARP inhibitor olaparib as a surrogate for “BRCAness”, we performed a CRISPR/Cas9-based genome-scale loss-of-function screen and identified TIGAR as a modifier of olaparib response. TIGAR knockdown increases the level of intracellular reactive oxygen species, induces more DNA damage after olaparib treatment, and causes “BRCAness” by downregulating BRCA1 and genes in the Fanconi anemia pathway. Further, TIGAR knockdown induces senescence, reduces spheroid tumor cell growth, and enhances the cytotoxic effect of olaparib. Finally, TIGAR is amplified in ovarian cancer and a higher expression of TIGAR is associated with poor overall survival in high-grade serous ovarian cancer. Our study shows that TIGAR knockdown causes extensive transcriptional reprogramming and enhances sensitivity to PARP inhibitor, demonstrating that TIGAR is a relevant therapeutic target for treating ovarian cancer.

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POOLED GENOMIC SCREENS IDENTIFY ANTI-APOPTOTIC GENES AS MEDIATORS OF CHEMOTHERAPY RESISTANCE IN OVARIAN CANCER

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Primary high-grade serous ovarian cancer (HGSOC) is often sensitive to platinum and taxane combination chemotherapy, but most patients relapse with chemotherapy-resistant disease. Although alterations in DNA repair function, gene expression, apoptosis, and other pathways have been described that can mediate chemotherapy resistance in HGSOC, the full landscape of HGSOC drug resistance mechanisms and the optimal strategies to eliminate resistant disease have not been fully elucidated. We performed systematic, unbiased near-genome-scale pooled overexpression and CRISPR/Cas9 knockout screens in two BRCA2-mutant HGSOC cell lines to identify genes promoting survival following cisplatin, paclitaxel, or cisplatin/paclitaxel treatment. Anti-apoptotic genes including BCL2L1 (BCL-XL), and BCL2L2 (BCL-W) were among the top hits mediating chemotherapy resistance in the overexpression screen. In the CRISPR/Cas9 screen, loss of pro-apoptotic genes (caspases, APAF1) conferred resistance, and knockout of BCL2L1 sensitized to platinum. A secondary overexpression screen of ~400 genes confirmed anti-apoptotic proteins BCL-XL, BCL-W and BCL-2 as top resistance genes, and validated numerous other candidates. Of note, anti-apoptotic genes BCL2L1 and MCL1 are focally amplified and overexpressed in patients with primary HGSOC. In HGSOC cell lines, overexpression of BCL-XL or BCL-W, and to a lesser extent BCL-2 or MCL1, conferred platinum and taxane resistance and decreased chemotherapy-induced apoptosis in HGSOC cell lines. We systematically tested small molecule inhibitors of BCL-2, BCL-XL, MCL1, or BCL2/BCL-XL as single agents or combined with chemotherapy in HGSOC cell lines. Inhibiting BCL-XL, MCL1, or BCL2/BCL-XL, but not BCL-2, significantly increased cell death when combined with cisplatin or paclitaxel. BCL-XL, MCL1, or BCL2/BCL-XL inhibitors also synergized with olaparib, a poly- ADP-ribose inhibitor. Concomitant overexpression of BCL-XL, BCL-W, or MCL1 abrogated the sensitizing effect of the anti-apoptotic protein inhibitors, depending upon the specific inhibitor. Taken together, unbiased near-genome-scale overexpression screens and patient genomic data highlight the role of the intrinsic pathway of apoptosis in HGSOC chemotherapy resistance. Our studies validate that anti-apoptotic proteins mediate resistance to several clinically relevant drugs in HGSOC, and support that BCL-XL and MCL1 may be therapeutic targets in HGSOC, particularly in combination with DNA-damaging agents.

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NOTES

IDENTIFICATION OF P53 PRIONS AS AN INDEPENDENT PROGNOSTIC MARKER FOR SURVIVAL IN HIGH-GRADE SEROUS OVARIAN CANCER

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PURPOSE: Although the discovery of prions was rewarded with a Nobel Prize, their existence was only attributed to a limited number of diseases. Recent evidence suggests that their role has been underestimated and several other proteins carry prion-like properties, like ß-amyloid, and most recently p53. High-grade serous ovarian cancers (HGSOC) harbor TP53 mutations in about 96% of cases. These mutations promote p53 aggregation, which might be responsible for complete abrogation of tumor suppressor functions, resulting in dominant-negative activity and oncogenic gain-of-function. Here, we describe the use of an ELISA-based technology for p53 prion detection in fresh-frozen tumor tissue and their clinical relevance in ovarian cancer.

EXPERIMENTAL PROCEDURES: Fresh-frozen tumor tissue specimens of 81 HGSOC patients, who previously had been included in the EU-funded OVCAD study, were analyzed. For each of these patients at least 5-year follow-up data were available. For the detection of p53 prions the Seprion-ELISA, previously designed for the detection of BSE and scrapie was adapted and optimized. To investigate the impact of p53 aggregation on clinical outcomes (overall survival and progression-free survival), we performed a complete-case analysis. In a subset of patients the Ki67 proliferation index and a homologous recombination deficiency (HRD) score, based on the Myriad score, were available. Correlations were analyzed using ANOVA and t-test.

RESULTS: In 39 of 46 (84.8%) patients with missense mutated cancers a p53 prion specific signal was observed. The aggregation propensity varied considerably within samples carrying the same mutations. Multivariable Cox regression models, with respect to other prognostic factors significantly associated with overall survival in patients with late-stage serous epithelial ovarian cancer (age, FIGO stage and presence of a residual tumor), show superiority of the group with extensive p53 aggregation in overall survival and in progression-free survival. No and moderate p53 aggregation are associated with a worse overall survival in contrast to high p53 aggregation (P values 0.025 and 0.011). Similar results in these groups are assessed for progression-free survival (P values 0.030 and 0.008). Interestingly, the group with extensive p53 aggregation was associated with a non-significant trend toward higher HR deficiency. Furthermore, this group had a significantly higher Ki67 index compared to patients with moderate p53 aggregation (P value 0.033).

CONCLUSIONS: This study reports the first specific and quantitative screening for p53 prions in patient material. We were able to demonstrate that the p53-Seprion-ELISA is a robust and highly sensitive detection tool for p53 prions. Our data show that the aggregation propensity is not only depending on the TP53 mutation and that other cofactors may be involved. Moreover, we show that p53 aggregation is an independent prognostic marker for survival. The higher Ki67 proliferation index and the trend towards higher genomic instability in patients with extensive p53 aggregation suggest that these tumors have an increased likelihood of response to platinum-containing therapy. To conclude, we demonstrated the high potential of p53 aggregation as a biomarker for patients' survival, suggesting that classification of patients based on the amount of aggregated p53 could allow therapy decisions.

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MODELING ENDOMETRIOID AND HIGH GRADE SEROUS CARCINOMAS IN THE MOUSE USING CRISPR/CAS9-MEDIATED SOMATIC GENE EDITING IN FALLOPIAN TUBE EPITHELIUM

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Genetically engineered mouse models (GEMMs) have contributed significantly to our understanding of the role of specific gene defects in human cancers. GEMMs that recapitulate many of the molecular and biological characteristics of various histologic subtypes of ovarian carcinomas have already been developed. However, conventional methods of GEMM development based on Cre-lox technology require the time-consuming and labor-intensive processes of transgenic line production and cross-breeding, a problem that is exacerbated when developing cancer GEMMs based on multiple genetic defects. The RNA-guided Cas9 nuclease from the microbial clustered regularly interspaced short palindromic repeats (CRISPR) adaptive immune system potentially offers a more rapid and versatile alternative platform for tumor modeling in the mouse, as it can be used to target multiple genomic loci simultaneously by specifying a 20-nt targeting sequence for each gene of interest. The development of gynecological cancer GEMMs using CRISPR/Cas9-mediated conditional gene editing has not yet been reported. We have tested this approach to generate endometrioid carcinoma (EC) and high-grade serous carcinoma (HGSC) in the mouse oviductal epithelium.

We first selected potentially optimal 20-nt sequences (guide RNAs) targeting the murine orthologs of the *Apc* and *Pten* tumor suppressor genes for the EC model, and the *Brca1, Trp53, Rb1,* and *Nf1* genes for the HGSC model. Efficient cutting mediated by each guide RNA was validated in vitro by the Surveyor nuclease assay in NIH3T3 mouse fibroblasts. Next, we generated two transgenic mouse lines: one carrying a transgene with guide RNAs targeting *Apc* and *Pten* in tandem as a single guide RNA (*sgAP*) and a second carrying a transgene with a single guide RNA targeting *Brca1, Trp53, Rb1*, and *Nf1* (*sgBPRN*). These transgenic lines were then crossed with *Ovgp1-iCre-ER*^{T2} and *Rosa26*$^{LSL\text{-}Cas9\text{-}EGFP}$ (Jackson laboratory) mice to allow for conditional (Tamoxifen-regulated) inactivation of *Apc-Pten* or *Brca1-Trp53-Rb1-Nf1* specifically in the FTE. Endometrioid carcinomas were identified in *Ovgp1-iCreERT2;Rosa26*$^{LSL\text{-}Cas9\text{-}EGFP}$*;sgAP* mice by 20 weeks post tamoxifen and early HGSC was present in an *Ovgp1-iCreERT2*; *Rosa26*$^{LSL\text{-}Cas9\text{-}EGFP}$*;sgBPRN* mouse 34 weeks post tamoxifen treatment. The tumors derived from Cre-CRISPR/Cas9-sgRNA technology showed similar morphology and immunophenotypic characteristics to tumors arising in the models using Cre-lox technology based on the same genetic defects. Insertions/deletions (Indels) in tumor DNA were found in all of the targeted genes near the expected cut-sites, and confirmed by Sanger sequencing of cloned PCR products. All of the Indels resulted in predicted premature protein truncation and loss of function. Our results show that *CRISPR/Cas9-sgRNA* system genome editing can be used successfully to model gynecological cancers in mice.

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CEREBELLIN-2 (CBLN2) IS DIFFERENTIALLY EXPRESSED IN OVARIAN CANCER SUBTYPES

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BACKGROUND: Cerebellin-2 (CBLN2) is a 24 kDa cytoplasmic, membrane and secreted glycoprotein that forms intracellular and extracellular homodimer and heterodimers. CBLN2's role in ovarian cancer pathogenesis has not been studied.

EXPERIMENTAL PROCEDURES: Serum-free conditioned media from twenty-two cell lines, represented by SV40 immortalized fallopian tube (FT-194, FT-190), SV40 immortalized ovarian surface epithelium (1816-575), low-grade serous adenocarcinoma (HEY), high-grade serous adenocarcinoma (TOV-1946), clear cell adenocarcinoma (TOV21G), cisplatin-sensitive high-grade serous adenocarcinoma (A2780-S) and cisplatin-resistant high-grade serous adenocarcinoma (A2780-CP), were prepared. Eight cell lines were submitted for isobaric tagging for relative and absolute quantification (iTRAQ®) differential expression analysis. Briefly, each of the eight secretomes were trypsin-digested and labeled with one of eight iTRAQ® 8-plex reagents and subsequently submitted for either two-dimensional capillary liquid chromatography direct data dependent peptide tandem mass spectrometry on an Orbitrap Velos system or one-dimensional mass spectrometry analysis. Western blot validation using an anti-CBLN2 antibody was used for protein validation.

RESULTS: Overexpression of CBLN2 in A2708-CP ranged from 6.4 to 12.3 times greater when compared to ovarian and fallopian tube control cell lines and 5.4 times greater when to compared to the cisplatin-sensitive cell line A2780-S. Western Blot demonstrated a differentially expressed 24 kDa band amongst subtypes.

CONCLUSIONS: CBLN2 is upregulated in the chemoresistant high grade serous carcinoma cell line A2780-CP compared to normal fallopian tube and ovarian surface epithelial cell lines. These results are promising for a marker for chemoresistance and further experiments in serum validation and characterization are warranted.

The views expressed in this abstract are those of the author and do not reflect the official policy of the Department of Army/Navy/ Air Force, Department of Defense, or U.S. Government.

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UPREGULATION OF MIR-328 CONTRIBUTES TO OVARIAN CANCER STEM CELL MAINTENANCE BY DOWNREGULATING DDB2

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Cancer stem cells (CSCs) are a particular subpopulation of cells that are characterized by self renewal, differentiation and enhanced tumorigenicity. They are responsible for tumor metastasis, relapse and development of drug resistance. Thus, eradication of CSCs is essential for improved patient prognosis. Micro RNAs are a group of small non-coding, endogenous RNAs that are found to regulate cancer stem cell characteristics by binding to mRNA in a sequence specific manner. In ovarian cancers, a wide array of Micro RNAs have been found to show differential expression of which miR328-3p deserves special mention. In this study, a Micro RNA Nanostring profile analysis reveals a significant upregulation of miR-328-3p in ovarian cancer stem cells isolated from both ovarian cancer cell lines and primary ovarian tumors as compared to their corresponding bulk cells. Moreover, it was found that inhibition of miR-328 limited the CSC population in ovarian cancer cells whereas overexpression of miR-328 enriched the CSC population, thus accounting for miR-328 as an onco-miRNA. The upregulation of miR-328 not only increased the percentage of ALDH+ cells in ovarian cancer bulk cells, but also increased the tumorigenicity and sphere formation ability. This was supported by the orthotopic ovarian xenograft assay. Further investigation revealed that reduced phosphorylation of Erk in ovarian cancer stem cells owing to reduced levels of Reactive Oxygen species (ROS) could be a prospective mechanism behind elevated miR328 expression and maintenance of CSC characteristics. Inhibition of phosphorylated Erk expression in ovarian cancer bulk cells by use of commercially available Erk inhibitor, U0126, led to a significant increase in miR328 expression. Simultaneously, upregulation of phosphorylated Erk in ovarian cancer stem cells not only reduced miR328 expression, but also displayed a significant reduction in expression of cancer stem cell markers (Oct4, Sox2, Nanog), sphere formation ability and tumorigenesis. We obtained a similar trend of results on regulating the expression of pErk by use of Reactive Oxygen Species to ovarian cancer cells. These data further helped us confirm our speculation that reduced ROS promotes the maintenance of CSCs characteristics through inactivation of Erk signalling pathway. Besides, we also identified DDB2 as a direct target of miR328. Our previous findings demonstrate that DDB2 is able to limit ovarian CSC population by disrupting their self renewal capacity. Thus, we conclude that elevated miR328 in ovarian CSCs, resulting from inactivated Erk1/2 activity, is responsible for maintenance of stemness by inhibition of DDB2 expression. Targeting miR-328 could therefore be a novel therapeutic strategy to eradicate CSCs in ovarian cancer.

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CD73: A NEW DRIVER AND A THERAPEUTIC TARGET IN OVARIAN CANCER STEM CELLS

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Many biological and clinical features of ovarian cancer (OC) support the notion that the disease is driven by a subpopulation of self-renewing cancer stem cells (CSC) that are able to generate the entire progeny of short-lived, differentiated and heterogeneous cells that compose the tumor bulk. Ovarian cancer stem cells (OCSC), in particular, have been proposed to drive and sustain tumor dissemination, recurrence and chemoresistance, most likely due to their slow cycling rate and their detoxifying molecular machineries. This provides the rationale for investigating OCSC as suitable targets for OC eradication.

Our study aims at assessing the functional contribution of OCSC to OC etiology and progression and at defining their molecular and functional traits. Specifically, we are pursuing these objectives through the accomplishment of the following milestones: 1) collection of normal and pathological samples; 2) identification of OCSC based on functional properties; 3) molecular profiling of OCSC and their normal counterpart; 4) characterization of novel genes/pathways involved in OCSC function (clonogenicity, tumorigenicity, quiescence, chemoresistance, etc.).

We have established a repository of patient-derived primary cells isolated either from OC or from its tissues of origin, ovarian surface or fallopian tube epithelium. The OCSC subpopulation and their normal counterpart were then derived from primary cultures and used to profile their specific transcriptome, inferring a OCSC signature. Among the genes differentially expressed in OCSC, we focused on CD73, which encodes a membrane-associated 5'-ectonucleotidase that accounts for the generation of extracellular adenosine. Genetic ablation experiments revealed that CD73 acts as a driver of OC cell stemness and tumor initiation. Furthermore, the pharmacological inhibition of CD73 reduced OCSC self-renewal and tumorigenesis, highlighting the druggability of CD73 in the context of OCSC-directed therapies. The biological function of CD73 in OCSC required its enzymatic activity and involved adenosine signaling. Mechanistically, CD73 promotes the expression of stemness and epithelial-mesenchymal transition-associated genes, implying a regulation of OCSC function at the transcriptional level.

In summary, CD73 is involved in OCSC biology and may represent a therapeutic target for innovative treatments aimed at OC eradication. In this context, CD73 is involved in tumor immune escape and actively investigated as a target to restore antitumor response. Thus, CD73-targeted therapies may combine the benefit of OCSC neutralization with overcoming CD73-driven immune escape, resulting in a synergistic effect towards OC eradication. Furthermore, CD73 neutralization might help preventing OCSC-dependent chemoresistance, thus enhancing the therapeutic efficacy of conventional chemotherapy. The therapeutic potential of CD73-based treatments and the molecular function of CD73 in OCSC are currently under investigation.

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MEK INHIBITOR TRAMETINIB IN HIGH GRADE OVARIAN CANCER PROLIFERATION

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A major challenge in ovarian cancer therapy is very high recurrence that is currently attributed to the presence of cancer stem-like cells (CSCs). CSCs are characterized by pronounced chemoresistance and high clonogenic ability that allow them to survive treatment and trigger relapse. The search for new treatment approaches capable of eradicating CSCs is crucial to prevent recurrent disease.

Despite lack of BRAF or KRAS mutations in high grade serous ovarian cancer, MEK1/2-ERK1/2 pathway is activated in ovarian cancer cell lines and primary tumors. The aim of present work was to evaluate MEK inhibitor trametinib on cell proliferation and tumorigenesis. Trametinib is approved by FDA for melanoma and other solid tumors with specific BRAF mutations, thus can be used in a timely manner in clinic if found effective.

We used cisplatin-resistant OVCAR8 and PEO4 cell lines displaying very high fraction of CD133-positive cells exhibiting CSC-like properties as main experimental model. Treatment of cells with trametinib caused very efficient inhibition of MEK-ERK pathway activity (up to 100% inhibition of ERK1/2 phosphorylation). Inhibition of pathway activity was confirmed by MEK-ERK-associated genes downregulation as assessed by RT-qPCR. ***PHLDA1***, ***SPRY2*** and ***DUSP4*** genes were identified as early MEK responders whereas ***SPRY4***, ***ETV4*** and ***ETV5*** were responsive to prolonged MEK inhibition.

Trametinib treatment resulted in significant reduction of cell growth rate and number of viable cells (5-fold reduction with 100nM trametinib, $p<0.05$) in both cell lines. Cell cycle assay demonstrated cell cycle arrest in G1/0 -phase indicating that MEK-ERK activation is required for transition from G1 to S-phase. To further elaborate associations between MEK pathway activity and cell proliferation, we studied cells enriched in different stages of S- and G2-phase. MEK pathway activity was subject to prominent activation during S-phase reflected by significant increase in phosphorylation levels of ERK1/2, its direct targets FRA1 and p90RSK and subsequent upregulation of reporter genes ***PHLDA1***, ***SPRY2*** and ***DUSP4.***

Our data indicate that trametinib treatment may induce efficient inhibition of ovarian cancer cell proliferation and therefore may have positive impact upon duration of remission for ovarian cancer patients. While trametinib alone is not capable of eradicating ovarian CSCs, its combinations with other drugs may provide a promising tool for ovarian cancer treatment, especially for tumors with high level of MEK-ERK pathway activity. One of such drugs to use in conjunction with trametinib can be Aldehyde Dehydrogenase (ALDH) inhibitor 673A that was previously characterized in our research as a compound capable of inducing cell programed necroptosis in ovarian CSCs.

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CELL OF ORIGIN, MUTATION AND MICROENVIRONMENT: MODELING EARLY EVENTS OF ENDOMETRIOSIS ASSOCIATED CANCERS

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Both clear cell ovarian carcinoma (CCOC) and endometrioid ovarian carcinoma (ENOC) are associated with ovarian endometriotic cysts, which is believed to be their precursor lesion. However, genomic evidence is lacking which could explain how these two clinically distinct histotypes of ovarian cancer arise from the same precursor lesion. We therefore hypothesized that these cancers arise from distinct cells of origin within endometrial tissue. Global proteomic analysis of ovarian cancer histotypes identified CTH as a marker for CCOC. We further found that CTH is highly expressed in the ciliated cells of endometrium (both ectopic endometrium and endometriosis), and of the fallopian tube, with little expression in the secretory cells. We also find that other ciliated cell markers are expressed in CCOC, whereas endometrial secretory cell markers are expressed in ENOC. We propose a new model of CCOC and ENOC histogenesis wherein ENOC is derived from cells of secretory cell lineage whereas CCOC is derived from cells of ciliated cell lineage. However, it remains unclear how external factors in the endometriotic cyst cooperate with cell of origin and mutation to promote cancer formation. To study normal tissue biology, we are using organoid cultures of normal endometrium. As ciliated cells of the endometrium are rare, and we have a particular interest in determining whether they have other features that may link them to CCOC, we used a Notch inhibitor, DBZ, to force ciliated cell differentiation in the organoids. We observed a dramatic shift in the cellular content with DBZ treamtment towards ciliated cells. We performed single cell RNA sequencing (scRNAseq) on these endometrial organoids. In the normal endometrial organoids, cells were predominantly a secretory phenotype, characterized by high ESR1 expression, with a minor ciliated cell population. The ciliated cell population expressed several known ciliated markers (FOXJ1 and DNAH12). Upon treatment with DBZ, the number of secretory cells decreases dramatically and two populations of cells emerge which have ciliated cell markers. The larger ciliated cell population is similar to the ciliated cells in the untreated organoids. The smaller ciliated cell population in the DBZ treated organoids express some ciliated cell markers, but clusters separately from normal ciliated cells. We believe this population may reprepsent an intermediary population, which has not fully differentiated. Interestingly, this population expresses the cytokine IL6, while the normal ciliated cell population does not. This is of note because CCOCs express more IL6 compared to the other histotypes. Therefore, we can speculate that this intermediary ciliated cell population may represent cells from which CCOC arise, however more testing is needed. In the future, the scRNAseq data from organoids will be compared to CCOC and ENOC tumors to determine whether the tumors resemble more closely one population of normal cells. We will use viral transduction to introduce mutations into the organoid cultures to determine whether specific mutation leads to transformation towards a CCOC or ENOC-like phenotype. These studies will enable us to tease apart the relative contribution of mutation, microenvironment and the cell of origin to promote tumor formation.

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UTILIZATION OF PATIENT-DERIVED TUMOR XENOGRAFT (PDX) MODELS IN ONCOLOGY

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BACKGROUND: The 5-year survival rate of patients with recurrent epithelial ovarian cancer is only 30%, in part due to relapse and resistance to first-line platinum-based chemotherapy. Even with marked progress toward understanding ovarian cancer biology, the translation of research findings into new therapies is still an enormous barrier to progress. Recent data suggests a 90% failure rate for new oncology drugs in the clinic. Development and preclinical testing of new cancer therapies has been limited by the availability of clinically relevant models that recapitulate the molecular and phenotypic characteristics of primary ovarian cancers. To overcome this barrier, we have initiated collaborative project between Stephenson Cancer Center (SCC) and Oklahoma Medical Research Foundation (OMRF) with the goal to generate a biobank of patient-derived xenograft (PDX) models derived directly from ovarian cancer patients undergoing therapy.

RESULTS: PDX tumor models are generated in the Patient-Derived Xenograft and Preclinical Therapeutics (PDX-PCT) Core at OMRF and used to improve preclinical evaluation of new drugs towards more personalized medicine. We have collected blood samples and tumor tissues from consenting patients having primary debulking surgery at Stephenson Cancer Center since 2015 (>140 unique patients). Fresh tumor tissue is minced into small tumor chunks (~2 mm) and implanted into immunocompromised mice. PDXs are expanded for 3-4 passages and characterized based on tumor type, histology and molecular characteristics. Immunohistochemical and molecular analysis showed that histology of the original patient tumors as well as the expression of commonly used markers for high-grade serous ovarian cancer such as cytokeratin, PAX8 and WT1 are perfectly maintained in the derivative PDX lines. Genotyping of patients' tumors and their corresponding PDXs using Illumina's Exome Array revealed that single nucleotide polymorphism (SNP) profile found in the original tumors is perfectly maintained in PDX lines. One of the PDX limitations is that human tumor stromal and immune cells are being replaced with mouse equivalents upon in vivo propagation of PDXs, which negatively affects quality of genomic or proteomic analyses. To overcome this challenge, we optimized a method to deplete mouse cells from PDX tumors by magnetic cells separation. Our data showed that ovarian cancer PDXs contain around 28-54% of human tumor cells, where the rest are mouse cells. We also optimized protocols to utilize pure population of human tumor cells isolated from PDXs to transduce these with lentiviral vectors expressing GFP and/or luciferase to generate luciferized PDXs. Luciferized ovarian PDXs are excellent models used for non-invasive orthotopic tumor growth and drug response monitoring.

CONCLUSIONS: Collection of our PDXs illustrates heterogeneity and diversity of human ovarian tumors. However, each PDX maintains essential molecular features of the original patients' tumor. Our goal is to generate clinically faithful ovarian tumor models that will provide a platform to learn more about cancer biology and to screen these for new better therapies.

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IDH1 IS A PRO-SENESCENT THERAPY IN HIGH-GRADE SEROUS OVARIAN CANCER

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Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer. High-grade serous carcinoma (HGSC) is the most frequently diagnosed and fatal histosubtype of EOC. We found that HGSC cell lines exhibit increased TCA cycle metabolites compared to fallopian tube cells, the proposed site of HGSC origin. Therefore, we propose that inhibition of TCA cycle metabolism may be a novel therapeutic strategy for HGSC. To determine which TCA cycle metabolism enzymes are dysregulated in ovarian cancer, we performed an unbiased qPCR screen of all 27 enzymes in the pathway. Our results indicate that isocitrate dehydrogenase I (*IDH1*) expression is significantly altered in EOC cell lines compared to fallopian tube cells. IDH1 catalyzes the conversion of isocitrate to alpha ketoglutarate (αKG) in a reversible reaction. It is well-known that IDH1 mutations play a role in cancer; however, recent publications suggest wildtype IDH1 overexpression promotes primary glioblastoma progression in the absence of mutation. Wildtype IDH1 and its role in metabolism and epigenetics has never been investigated in HGSC.

To determine whether IDH1 plays a functional role in HGSC, we used both genetic and pharmacological approaches to suppress IDH1 in multiple HGSC cell lines. Knockdown or inhibition of IDH1 using the small molecule inhibitor GSK864 significantly decreased HGSC cell proliferation. Mechanistically, this was due to induction of senescence, a stable cell cycle arrest. We next aimed to determine the molecular mechanism underlying senescence induction due to inhibition of IDH1. Increased histone methylation of proliferation promoting genes (i.e., *CCNA2* and *PCNA*) is a characteristic of senescence. αKG acts as a cofactor for the Jumonji C (JmjC) histone demethylase family, suggesting that suppression of αKG may affect the methylation status of histones. αKG levels were significantly decreased in IDH1 knockdown cells. Therefore, we hypothesized that decreasing αKG levels in HGSC may modulate the epigenome to induce senescence. Chromatin immunoprecipitation (ChIP) experiments showed an increase in repressive H3K9me2 histone methylation at proliferation-promoting gene loci (*CCNA2* and *PCNA*) when IDH1 was knocked down. This correlated with a decrease in mRNA of both genes. These data suggest that knockdown of IDH1 induces senescence of HGSC cells by increased histone methylation of proliferation promoting genes.

Finally, we aimed to determine the JmjC demethylase family member that is inhibited by IDH1 knockdown. The JmjC KDM4 family modulates histone demethylation of histone H3 lysine 9. KDM4A is upregulated in EOC and correlates with worse overall survival. Interestingly, knockdown studies of KDM4A phenocopied those of IDH1 knockdown. Future studies are aimed to dissect the role of KDM4A in senescence induction. Altogether, these data suggest that targeting the metabolic enzyme IDH1 in HGSC induces senescence through epigenetic reprogramming and may be a novel metabolic therapy for HGSC patients.

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PATIENT DERIVED XENOGRAFTS (PDXS) AND PDX DERIVED TUMOR CELLS (PDTC) ALLOW THE IDENTIFICATION OF ACTIONABLE CANCER GENES AND TREATMENT OPTIONS FOR PLATINUM REFRACTORY/ RESISTANT OVARIAN CARCINOMAS

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Patients with advanced ovarian cancers have experienced little improvement in overall survival with standard treatments even after the incorporation of anti-angiogenic therapies. Besides anti-PARP inhibitors, matching individual critical genomic alterations with the best available drugs has not advanced as in other cancers, likely because a handful of cancer-related genes are mutated at high frequency, while many more are found mutated at much lower frequencies. This so called "mutation tail" is not only long but also mostly unexplored.

We used Patient Derived Xenografts (PDXs) to identify actionable cancer genes and PDX Derived Tumor Cells (PDTCs) to accelerate the discovery of treatment options. We envisioned that the alleged weakness of PDX models, i.e. lack of human stromal and immune cells, might be instrumental to identify mutations in cancer and to test approved or experimental targeted drugs as monotherapy or in different combinations to link biomarkers to treatments.

Forty-nine PDX lines from metastatic epithelial ovarian carcinomas have been propagated and fully characterized as far as histology, immunohistochemistry of epithelial and high-grade serous-specific markers and presence of TP53 and BRCA1/2 mutations.

Copy number variations (CNV) analysis and Whole Exome Sequencing (WES) were carried out PDX lines derived from naïve metastatic high-grade epithelial ovarian carcinomas, which came out to be refractory/resistant to platinum drugs. We studied non-synonymous mutations with allele frequencies ≥0.1. Only mutations in cancer genes listed in databases were further analyzed. SNPdb allowed ruling out polymorphisms. SIFT and PROVEAN softwares predicted deleterious or damaging effects onto the protein sequences. DGIdb helped selecting actionable genes.

We identified in one PDX line, a possibly loss-of-function mutation of the PIK3R1 gene (encoding the p85alpha regulatory subunit of PI3K) had an allele frequency=0.9 in early and late passages. Moreover, in two micro-dissected FFPE samples of the source tumor this mutation had an allele frequency nearly identical to that of the mutated TP53. Hence, PIK3R1W624R could be a trunk mutation in the PDX line and possibly in the human counterpart.

Treatment options were assayed ex-vivo, on short-term cultures of PDTCs of the PIK3R1W624R PDX line. Buparlisib, a pan-class I PI3K inhibitor, showed the ability to block proliferation of PDTCs and the growth in vivo of PDXs in regression preclinical trial. These data proofed-the-concept that a PDX-based pipeline is able to unveil actionable pathways for the treatment of advanced/ metastatic ovarian cancer.

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TARGETING THE FORMIN-ASSEMBLED ACTIN CYTOSKELETON AS AN ANTI-INVASION STRATEGY IN OVARIAN CANCER SPHEROIDS

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Due to high rates of recurrent metastasis during EOC progression, there is a critical unmet need to limit advancement of metastatic lesions present at diagnosis and upon initial recurrence. In non-hematogenous dissemination, cancer cells metastasize within the peritoneum and attach to visceral organs. Single cells, multi-cell clusters, or highly invasive multi-cellular spheroids are shed into peritoneal fluid from primary tumors, or are released by mechanical disruption of peritoneal metastases during cytoreductive surgery. Peritoneal EOC metastasis is not halted upon surgical removal of primary or metastatic tumors; it is a dynamic, longitudinal process of recurring tumors in the peritoneal cavity/pleural space. Recurrent peritoneal metastasis likely occurs via tumor cells suspended in the accumulating ascites fluid attaching to and invading the peritoneal mesothelium. Thus, EOC spheroids promote recurrent peritoneal metastasis and must be targeted for therapeutic intervention. We wished to assess the therapeutic utility of small molecules called intramimics (IMM) and Diaphanous-autoregulatory domain (DAD) in EOC spheroid peritoneal metastasis models. Both IMMs and DADs stimulate mDia2 formin function, and are thus classified as agonists. Formins are intracellular cytoskeletal nanomachines that assemble G-actin monomers into F-actin filaments. Formin-mediated F-actin assembly regulates stress fiber formation and generates forces to deform membranes into protrusive structures (i.e., lamellae, filopodia) to enable cell migration. While this process is necessary for normal cell migration (i.e., wound healing, development), it is abnormally invoked in metastasis. mDia proteins also support tumor cell-cell interactions by promoting the assembly of the F-actin architecture underlying cell-cell adherens junctions (AJs) that link cells of epithelial sheets together. IMMs and DADs specifically agonize mDia2 formins, keeping the nanomachine “on”, and in so doing, promote constitutive F-actin assembly within cells. Therefore, we hypothesize that mDia2 formin agonism would effectively block EOC spheroid invasion in 3D matrices by halting single cell invasive egress from spheroids. Using SKOV-3 and OVCA429 adenocarcinoma EOC cells, we demonstrated that mDia2 protein associates with proteins underlying AJ complexes. Specifically, mDia2 protein associates with alpha and beta catenins, yet not E- or N-cadherins. We further show that siRNA-mediated depletion of mDia2 alters the subcellular localization of AJ-associated proteins, impacting F-actin accumulation at the AJ and enhancing spheroid invasive dissemination. In contrast, mDia agonism using IMM and mDia DAD small molecules effectively inhibits EOC spheroid invasion, disallowing single cell egress from spheroids. Collectively, these results indicate a role for mDia formins in regulating invasive egress from EOC spheroids. Thus, there may be therapeutic utility to targeting mDia formin cytoskeleton effectors in halting EOC spheroid invasive egress.

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ERBB FAMILY GENOMIC ALTERATIONS IN GYNECOLOGICAL MALIGNANCIES

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Activation of HER2 or EGFR signaling is a common, targetable oncogenic mechanism in solid tumors such as breast, gastroesophageal, lung and colorectal carcinomas. In addition, ERBB3 activation has been shown to confer sensitivity to HER2-targeted therapies, and ***ERBB4*** mutation is reported a mechanism of resistance. From a database of 14,692 comprehensive genomic profiles for both common and rare gynecological malignancies, we extracted 9,121 ovarian tumor profiles and evaluated the prevalence of genomic alterations (GA) in the ***ERBB*** family (***EGFR, ERBB2, ERBB3, ERBB4***), including base substitutions, small indels, amplification (AMP), and rearrangements (RE).

At least 50ng of DNA was extracted from FFPE specimens and sequenced to high, uniform median coverage (>500X) by hybridization capture of exons from up to 406 cancer-related genes and select introns from up to 31 genes commonly rearranged in cancer. For some samples, RNA sequencing of 265 genes was also performed. Tumor mutational burden (TMB, mutations/Mb [mut/Mb]) was determined on ~1.1 Mbp of sequenced DNA, and microsatellite instability (MSI) and overall genomic loss of heterozygosity was evaluated for subsets of the samples.

Of 9,121 tumors, most were serous carcinomas (n = 6,847). A total of 615 (6.7%) tumors had ≥1 oncogenic GA in an ***ERBB*** gene, with 40 (0.4%) having ≥2 ***ERBB*** GA. ***ERBB***+ were most often mucinous (15.9%) or clear cell (13.9%) carcinomas. Serous carcinomas were ***ERBB***+ in 4.3-7.1% of samples. Patient age was comparable in both the ***ERBB***+ and ***ERBB***- tumors (median 61 y vs. 61 y, range 19-94 y vs. 2-95 y).

ERBB2 (HER2) AMP was the most common ***ERBB*** GA; 2.7% of serous carcinomas had amplified ***ERBB2***. GA distribution by ***ERBB*** gene was as follows: ***ERBB2*** 4.5% (n=394; of those AMP were 78.4%, n=309), ***ERBB3*** 0.8% (n=87; AMP 50.6%, n=44), ***EGFR*** 0.9% (n=90; AMP 45.6%, n=41), and ***ERBB4*** 0.6% (n=66; AMP 65.1%, n=43). Known or potential oncogenic rearrangements, fusions, and splice site GA were observed in both ***ERBB2*** (n=20) and ***EGFR*** (n=10), including a kinase domain duplication in EGFR and exon 16 deletions in ERBB2. Although ***ERBB2*** AMP is concordant with HER2+ by IHC or FISH, 70/615 (11.4%) HER2-driven samples harbored targetable, oncogenic GA that would not be detected by these methods, including the well-characterized A775_G776insYVMA (n=13), S310Y/F (n=10), and V842I (n=8).

In ***ERBB***+ tumors, the most commonly co-occurring GA affected ***TP53*** (76.6%), ***PIK3CA*** (19.5%), ***MYC*** (15.7%), ***TERC*** (14.6%), ***ARID1A*** (18.3%), and ***CCNE1*** (20.5%). Pathways likely to confer some level of resistance to ***ERBB***-targeted therapies were often mutated: PI3K-AKT-MTOR 35.0% (n=215), RAS-RAF-MEK 18.2% (n=112), and FGFR 5.5% (n=34). ***ERBB***+ tumors had slightly higher TMB scores: median 3.5 mut/Mb vs 2.6 mut/Mb, and average 6.7 vs 3.7 mut/Mb. Of 502 samples analyzed for microsatellite instability, 12 were MSI high. In conclusion, nearly 7% of ovarian tumors harbor potentially targetable GA in an ***ERBB*** family gene, including HER2 and EGFR. Of these, 49.6% of samples would not be positive by HER2 IHC or FISH. Further exploration into the efficacy of HER2- or EGFR-targeted therapies, and the effects of co-altered pathways, in ovarian carcinomas may be promising.

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AMPLIFICATION OF USP13 DRIVES OVARIAN CANCER METABOLISM

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Dysregulated energetic metabolism has been recently identified as a hallmark of cancer. Although mutations in metabolic enzymes hardwire metabolism to tumourigenesis, they are relatively infrequent in ovarian cancer. More often, cancer metabolism is re-engineered by altered abundance and activity of the metabolic enzymes. Here we identify ubiquitin-specific peptidase 13 (USP13) as a master regulator that drives ovarian cancer metabolism. USP13 specifically deubiquitinates and thus upregulates ATP citrate lyase and oxoglutarate dehydrogenase, two key enzymes that determine mitochondrial respiration, glutaminolysis and fatty acid synthesis. The USP13 gene is co-amplified with PIK3CA in 29.3% of high-grade serous ovarian cancers and its overexpression is significantly associated with poor clinical outcome. Inhibiting USP13 remarkably suppresses ovarian tumor progression and sensitizes tumor cells to the treatment of PI3K/AKT inhibitor. Our results reveal an important metabolism-centric role of USP13, which may lead to potential therapeutics targeting USP13 in ovarian cancers.

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DYNLL1 INHIBITS DNA END RESECTION IN BRCA1-DEFICIENT CELLS AND REGULATES PARP INHIBITOR SENSITIVITY

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High-grade serous ovarian carcinoma (HGSOC) patients with germline mutations in BRCA1/2 exhibit high sensitivity and improved outcome to double strand DNA break (DSB)-inducing agents [i.e. platinum and Poly(ADP-ribose) polymerase inhibitors (PARPi)] due to underlying defects in DNA repair via homologous recombination (HR). Due to their effectiveness, three PARP inhibitors (olaparib, rucaparib, niraparib) have recently gained FDA approval for the treatment of HGSOCs. However, de novo and acquired resistance to these agents is common even in the BRCA mutation carriers, and pose a significant, and unsolved, clinical challenge. Therefore, we adopted a systematic approach to comprehensibly identify unexplored factors/pathways that could be responsible for PARPi/platinum resistance in BRCA-defective HGSOC patients.

Here we identify DYNLL1 as a negative regulator of DNA end resection through a loss-of-function CRISPR screen in BRCA1-mutant ovarian carcinoma cells. DNA end resection is a vital process that initiates homologous recombination (HR)-mediated repair of double-stranded DNA breaks (DSBs), and consequently influences genome stability. In BRCA-defective HGSOC patients, DNA end resection is greatly compromised and contribute to the loss of HR and PARP inhibitor sensitivity. Loss of DYNLL1 allows DNA end resection and restores HR in BRCA1-mutant cells, thereby inducing resistance to platinum drugs and PARP inhibitors. In primary ovarian carcinomas low BRCA1 expression correlates with increased chromosomal aberrations, and the junction sequences of somatic structural variants indicate the loss of HR. Concurrent decrease in DYNLL1 expression in BRCA1 low ovarian cancers 'rescued' this phenotype with reduced genomic alterations and increased homology at putative lesions. DYNLL1 limits nucleolytic degradation of DNA ends by interacting with the DNA end resection machinery (MRN complex, BLM helicase and DNA2) in cells. The impact of DYNLL1 on end resection can be re-capitulated in vitro and this is dependent on direct interaction with MRE11. In the absence of exogenous stress, depletion of DYNLL1 slows DNA replication fork progression due to ectopic activity of MRE11. Therefore, we infer that DYNLL1 is an important anti-resection factor that significantly influences genomic stability and response to DNA damaging chemotherapy.

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VALIDATION OF RUNX3 AND CAMKIINα HYPERMETHYLATION AS PROGNOSTIC MARKER FOR OVARIAN CARCINOMA

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INTRODUCTION: Ovarian cancer is still the most lethal gynecological malignancy with a low five-years-overall survival rate. Except for BRCA1/2 mutations there is no predictive nor prognostic marker available indicating whether a patient is in need and eligible for personalized therapy. In a previous genome-wide array study we identified different methylation patterns in type II epithelial ovarian cancer patients with either good or poor outcome (PFS < vs. > 3 years). Using methylation-specific PCR (MSP) five marker candidates showed high prognostic value whereby the marker combination RUNX3/CaMKIINα was the most promising one [Häfner et al., 2015]. Moreover, in vitro experiments provided evidence that a transcript variant of RUNX3 can mediate Cisplatin sensitivity, further underlining the clinical relevance of this gene [Heinze et al., 2018].

METHODS: In this study we aim to validate the previous described prognostic value of the five candidates, especially of RUNX3 and CaMKIINα via MSP. Therefore, 100 fresh-frozen primary type II EOC samples from two different surgical centers were tested. Furthermore, previously analyzed samples were retested to gain insight on the marker distributions throughout the tumor and possible heterogeneity. The DNA was isolated via phenol/chloroform or QiaAmp Kit, converted to bisulfite DNA and tested via optimized MSP. All samples had similar clinical parameter (histology, FIGO grade, chemotherapy).

RESULTS AND DISCUSSION: By re-testing a subset of EOC samples previous results could been confirmed. Additionally, the methylation pattern was similar after renewed sectioning for DNA extraction pointing towards little tumor heterogeneity in the analyzed samples. Interestingly the methylation rate is not as high as expected from the initial analyses. Statistical analysis of the data is currently ongoing.

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MITOCHONDRIAL STRESS RESPONSE ADAPTATIONS ARE REQUIRED FOR OVARIAN CANCER ANCHORAGE-INDEPENDENT SURVIVAL AND METASTASIS

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Late stage ovarian cancer is marked by poor patient survival and significant metastatic spread throughout the peritoneal cavity. During transcoelomic spread cells must adapt to survive anchorage-independence and to cope with stress associated with matrix detachment and the hostile environment of the ascites. Our findings demonstrate that an important ovarian cancer cell adaptation during anchorage-independence is the up-regulation of the mitochondrial stress response proteins, the mitochondrial antioxidant enzyme superoxide dismutase 2 (Sod2), and it's regulator, the nutrient and redox sensing deacetylase SIRT3. We show that these proteins are necessary for anchorage-independent cell survival and transcoelomic metastasis *in vivo*. In addition to Sod2's role as a mitochondrial superoxide scavenger, mechanistic data show that Sod2 shifts the redox landscape of cancer cells to a higher hydrogen peroxide (H_2O_2) steady-state. This novel non-canonical function of Sod2 results in H_2O_2-dependent oxidation and inactivation of phosphatases, including PTEN. Sod2/H_2O_2-dependent redox signaling may hence be a novel driver of anchorage-independent survival and an important contributor to the high frequency of Akt activation observed in metastatic ovarian cancers. While these sub-lethal increases in cellular H_2O_2 lead to pro-tumorigenic signaling, this also places cells closer to the cytotoxic threshold of H_2O_2, making cells with high Sod2 expression exquisitely sensitive to exogenous H_2O_2 application, and H_2O_2 generating agents, including high dose ascorbate. These findings provide an important clue to the vulnerability of Sod2 expressing cancer cells. Our findings also demonstrate that SIRT3 is an important regulator of Sod2 activity during anchorage-independence, which challenges previous studies demonstrating that SIRT3 is a tumor suppressor, and points to a novel pro-survival role for SIRT3 during ovarian cancer spread. Given that SIRT3 is sensitive to nutrient stress, this regulation has further consequences on the ability of ovarian cancer cells to survive potentially nutrient deprived tumor environments. Our study has identified two important mitochondrial stress response genes that are specifically increased in response to matrix detachment and required for anchorage independent survival and metastatic spread of ovarian cancer. Our ongoing studies are investigating their role in the context of redox and nutrient stress of ascites and targeting these stress adaptations for therapeutic intervention.

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MODELS AND ANALYTIC TECHNIQUES OF MULLERIAN TISSUE-DERIVED ORGANOIDS

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INTRODUCTION: Ovarian cancer is the 5th deadliest cancer found in women and is the deadliest involving the gynecological tract. Most epithelial ovarian cancers have extra-ovarian origins and can be stratified into various histotypes: high and low-grade serous (HGS and LGS), endometrioid (ENOC), clear cell (CCOC), and mucinous – each of which are proposed to have distinct precursor lesions. We present organoids as a useful model to study precursor lesions and the process of tumorigenesis in epithelial ovarian carcinomas. Organoids recapitulate the ***in vivo*** growth microenvironment and are useful to study the mechanisms of tumorigenesis from healthy cells. We have previously proposed that ENOC arise from the secretory cell lineage, while CCOC originate from the ciliated cell lineage, and organoids are an ideal model to examine in greater depth the impact of mutation on specific cell populations, such as ciliated cells.

METHODS: Surgical fallopian tube and endometrial tissues, removed for non-cancer reasons, were cultured in 2D followed by plating into Matrigel. Matrigel cultures were supplemented with media containing stem/progenitor differentiation factors promoting organoid growth. To study the effect of mutations often found in ovarian cancers on organoid growth and development, gene knockouts were produced using CRISPR lentiviruses on cells prior to Matrigel culture. Lentiviral transductions were optimized for organoid formation and for minimizing invasiveness accrued on cells. CRISPR gRNA constructs were validated by Western Blot and qPCR. Organoids containing knockouts of p53, BRCA1 and BRCA2 were used to model precursor lesions of HGS, whereas ARID1A knockouts and an inducible PIK3CA activating mutations were used to model CCOC.

To gain further insight into ciliated cells of the endometrium, organoids were treated with the notch inhibitor-DBZ to drive differentiation of cells towards a ciliated cell lineage. We analyzed organoids by single-cell RNA sequencing (scRNA-seq), immunohistochemistry (IHC), and immunofluorescence staining (IF). Single cells were derived by purifying the organoids from Matrigel followed by a chemical and physical digestion. scRNA-seq was performed utilizing the 10X Genomics Platform and analyzed by in-house bioinformaticians. Bioinformatic analyses included stringent QC to remove low-quality and dead cells, before applying unsupervised learning algorithms like PCA and Gaussian mixture modeling as well as differential expression analysis to understand both how samples relate to each other and cell types discovered within each sample.

RESULTS: We successfully recapitulated the histology observed in tissues by growing endometrial and fallopian tube organoids. The notch inhibitor, DBZ forced ciliated cell differentiation, as observed by IHC, IF and scRNA-seq. scRNA-seq clustering of DBZ-treated organoid cultures revealed a possible intermediary state between progenitor and ciliated cells. Initial IHC and IF analyses of CRISPR-mediated organoids reveal successful gene manipulation.

CONCLUSIONS: Organoid cultures present as a powerful method for modelling precursor lesions; they can be readily manipulated genetically and with rapid turnaround compared to conventional mouse models. Organoids are also amenable to sequencing at single-cell resolution. The ability to model ovarian cancers with permanent knockouts in human tissue serves as a necessary link between animal models and human therapy.

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CLINICAL PATHOLOGIC EXPRESSION OF CELL CYCLE REGULATORY COMPLEXES IN HIGH GRADE SEROUS OVARIAN CARCINOMA

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INTRODUCTION: Cell cycle control is an important determinant of cancer progression and treatment response. Two key transcriptional regulatory complexes, DREAM and MMB, ensure coordinated cell cycle dependent gene expression. Though these complexes contain the same protein core called MuvB, they have opposing functions. The DREAM (DP, RB-like, E2F, and MuvB) complex represses over 800 cell cycle genes in G0/G1 and the MMB (Myb-MuvB) complex promotes mitotic gene expression. High expression of B-Myb, an oncogenic transcription factor involved in the MMB complex, is associated with cell cycle deregulation and poor prognosis in several cancers, including ovarian cancer. High B-Myb expression disrupts DREAM formation in human cell lines, resulting in increased proliferation. Previous analysis of TCGA data showed that MYBL2 (encoding B-Myb) undergoes gene copy number gain in 55% of high grade serous ovarian cancer (HGSOC) tumor samples and is associated with poor overall survival. We sought to validate these findings with clinical specimens and to investigate the role of DREAM- and MMB-regulated gene expression in HGSOC patient outcomes.

METHODS: We used expression levels of DREAM-and MMB-controlled genes as a functional readout for the status of these opposing transcriptional regulators. This retrospective study utilized tissue bank surgical pathology and cytology samples taken from 52 HGSOC lesions. RT-qPCR gene expression analysis was correlated to clinical and pathologic findings. Demographic information, follow-up, treatment, and outcomes data (age, Stage, optimal debulking, platinum sensitivity, survival) was obtained by chart review. Analyses of TCGA datasets were conducted in parallel.

RESULTS: RT-qPCR analysis of DREAM target genes (AURKA, KIF23, CCNB2, and FOXM1) revealed positive and significant correlations between MYBL2 and all genes tested: AURKA (ρ=0.4114, p<0.01), KIF23 (ρ=0.4953, p<0.001), CCNB2 (ρ=0.3278, p<0.05), and FOXM1 (ρ=0.5033, p<0.001). Stage at diagnosis, optimal debulking, platinum sensitivity and survival did not associate with high or low expression of target genes. High FOXM1 expression was associated with longer progression free survival (p<0.01). High CCNB2 (encoding cyclin B2) showed a trend (p=0.0643) with decreased overall survival, with a median time difference of 20 months between high (26 months) and low (46 months) groups. Analysis of TCGA HGSOC datasets revealed that DREAM and MMB target genes were significantly upregulated in the presence of high B-Myb expression (Fisher's exact test, p<0.01). The top 49 upregulated genes associated with high MYBL2 in HGSOC analysis have been previously annotated as DREAM target genes (χ2 with Yates correction p<0.001).

CONCLUSIONS: Increased expression of selected cell cycle genes correlates to increased formation of MMB, and reduced DREAM assembly in HGSOC tissue. High expression of MYBL2 is associated with deregulated cell cycle gene expression programs in HGSOC. Larger scale studies would clarify the clinical prognostic value of the DREAM- and MMB-regulated gene expression.

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UNRAVELLING THE STEROID METABOLOME IN EPITHELIAL OVARIAN CANCER

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INTRODUCTION: Ovarian tissue possesses the capacity for steroidogenesis from as early as seven weeks gestation. Perturbations in the steroidogenic pathways have been implicated in the development and progression of epithelial ovarian carcinoma in adulthood but the evidence has been limited. Analysis of RNA-seq data from ovarian tissue now allows the genes involved in these pathways to be studied comprehensively helping direct future investigations and highlighting possible mechanisms of the disease.

METHODS: RNA-seq data from 293 serous epithelial ovarian carcinomas from The Cancer Genome Atlas were extracted and the genes involved in steroidogenesis were systematically interrogated for high and low expression. Pathway analysis was employed to confirm significant findings. Comparison was made with novel RNA-seq data from first trimester fetal ovary and healthy adult ovary.

RESULTS: Genes involved in the synthesis of androgens (HSD17B1, SRD5A1, AKR1C3, AR and STS) were all significantly upregulated in the high grade serous ovarian carcinoma group when compared to normal healthy ovary ($p<0.05$).

Expression of several steroidogenic genes in malignancy appeared to mimic increased fetal expression with relative quiescence noted in the healthy ovary group.

CONCLUSIONS: Transcriptome analysis shows upregulated gene expression of the androgen synthesis pathway is associated with epithelial ovarian cancer. This is supported by immunohistochemistry findings in recent studies. Investigation of the androgen pathway does remain a valid opportunity for mechanistic insight into this disease and potentially diagnostic and therapeutic exploitation.

Ovarian steroidogenic gene expression in the first trimester appears to correlate with neoplastic growth in serous ovarian cancer but the significance of this is uncertain.

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CONDITIONAL BRCA2 SWITCH IN HUMAN CELLS TO STUDY TUMOR PROGRESSION

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The BRCA2 (Breast Cancer Susceptibility 2) gene is a caretaker of genome integrity. Germline mutations in BRCA2 predispose individuals to a high risk for ovarian and breast cancer. The BRCA2 protein plays an important role in repair of DNA double-strand breaks (DSBs) by homologous recombination. The hallmarks found in BRCA2 mutant tumors involve: genome instability including chromosome aberrations, sensitivity to cross-linking agents, presence of micronuclei, an abnormal number of centrosomes, and severe defects in homologous recombination. However, the initial steps of tumor progression in BRCA2 carriers remain elusive as the majority of cellular models are derived from tumor cell lines that have undergone prior selection in an environment of genomic instability. Here, we present the generation of isogenic, inducible BRCA2 human fallopian tube cell lines providing a model to study the acute loss of BRCA2 protein in the setting of initiation and progression towards tumorigenesis. In these otherwise normal human somatic cells, we have found that acute loss of BRCA2 impinges upon viability as well as other cellular abnormalities that may drive the accumulation and tolerance for further somatic mutations. We have discovered copy number variations modulated by crosslinking compounds in our inducible BRCA2 human fallopian tube cells. We are analyzing both the genetic and biochemical functions of BRCA2 in these processes. Our long-term goal is to elucidate the underlying molecular mechanisms that drive and sustain tumor initiation in the absence of normal BRCA2 function.

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CO-REGULATION AND FUNCTIONAL COOPERATIVITY BETWEEN FOXM1 AND RHNO1 BIDIRECTIONAL GENES IN HIGH-GRADE SEROUS OVARIAN CANCER

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PURPOSE: The FOXM1 transcription factor is canonically involved in cell cycle progression, but also promotes DNA repair and drug resistance, via its transcriptional activity. We previously reported that FOXM1 is located within the Ch.12p13.33 amplicon, which sustains copy number gains in ~60% of high-grade serous ovarian cancer (HGSC). More recently, we discovered that FOXM1 is arranged in a head-to-head configuration with RHNO1, a gene involved in the ATR/CHK1-dependent DNA replication stress (DRS) response. By virtue of its role in the DRS response, RHNO1 promotes homologous recombination (HR) DNA repair. We hypothesized that FOXM1 and RHNO1 are co-expressed in HGSC due to their bidirectional configuration, and that they functionally cooperate to promote HGSC cell survival, HR repair, and PARPi resistance.

METHODS: We used 5' RACE, reporter constructs, and CRISPR-Cas9 (CRISPR) to characterize the FOXM1-RHNO1 bidirectional promoter. We correlated FOXM1 and RHNO1 mRNA expression and copy number in normal tissue and cancer data, including single cell RNA sequencing (scRNA-seq) of immortalized fallopian tube epithelial (FTE) cells and HGSC cells. We used RNAi and CRISPR to disrupt FOXM1 and/or RHNO1 in HGSC cells, and determined the resulting impact on gene expression, cell cycle, cell survival, HR repair, and olaparib (PARPi) sensitivity.

RESULTS: FOXM1 and RHNO1 mRNA expression significantly correlated in normal tissues, including FTE, and in HGSC tumors and cell lines, both in bulk cells/tissues and at the single cell level. Co-expression appeared to result from the FOXM1-RHNO1 bidirectional promoter, which showed similar activity in each direction in cell models and correlated with endogenous mRNA expression. FOXM1 knockdown in HGSC cells led to enriched gene expression signatures for the G2/M checkpoint and reduced clonogenic survival. RHNO1 knockdown attenuated ATR/CHK1 signaling, reduced HR repair efficiency, and reduced HGSC clonogenic survival. Dual targeting of FOXM1 and RHNO1 using shRNA caused an additive effect on HR repair and clonogenic survival and caused synergistic sensitization of HGSC cells to olaparib treatment. Critically, bidirectional promoter targeting via a CRISPR-KRAB repressor recapitulated the effects of shRNA knockdown. Finally, FOXM1/RHNO1 bidirectional promoter repression led to olaparib sensitization in an in vitro HGSC model of acquired olaparib resistance.

CONCLUSIONS: We demonstrate that FOXM1 and RHNO1 are frequently co-expressed in HGSC, most likely due to coordinated activity from their bidirectional promoter. Importantly, the FOXM1/RHNO1 bidirectional unit exhibits functional cooperativity for HR DNA repair and cell survival, and synergistically promotes olaparib resistance. Based on our data, we suggest that functionally interactive bidirectional gene modules are an under-appreciated oncogenic mechanism. Finally, we suggest that FOXM1 and RHNO1, or their associated pathways, are promising combinatorial therapeutic targets in HGSC.

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TARGETING SPSB1 SENSITIZES PLATINUM-RESISTANT OVARIAN CANCER CELLS TO CISPLATIN

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OBJECTIVE: Emergence of chemoresistance remains a critical clinical issue responsible for treatment failure in patients with ovarian cancer. The precise mechanisms underlying the development of chemoresistance are not fully understood. In our previous study using methylomic and transcriptomic analyses, SPRY domain-containing SOCS box protein 1 (SPSB1) was found to be differentially expressed between cisplatin-sensitive and –resistant ovarian cancer cells. Here, we investigated the roles of SPSB1 as a new therapeutic target for ovarian cancer cells resistant to cisplatin.

METHODS: Knockdown of SPSB1 gene was performed by transfection of SPSB1 siRNA. Ovarian cancer cell lines were cultured for 5 days and 12 days after transfection for MTT assay and colony forming assay, respectively. Protein level was analyzed by western blot analysis. Cell migration assay was performed using Boyden chamber method.

RESULTS: Knockdown of SPSB1 using RNAi method dramatically inhibited cell viability and migratory capability of ovarian cancer cells. Also, SPSB1 knockdown significantly induced CDK inhibitors, p21 and p27 and inhibited anti-apoptotic Bcl-2, indicating that SPSB1 inhibition induces cell cycle arrest and apoptosis in ovarian cancer cells.

CONCLUSION: Our results suggest that SPSB1 might be a potential therapeutic target for cisplatin-resistant ovarian cancer.

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COMPREHENSIVE GENOMIC, PROTEOMIC, AND EXPERIMENTAL CHARACTERIZATION OF OVARIAN CLEAR CELL CARCINOMA CELL LINES FOR IMPROVED DRUG DEVELOPMENT

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PURPOSE: Ovarian cancer is a heterogeneous disease with multiple histological subtypes and a wide range of genetic aberrations. While high-grade serous carcinoma (HGSC) is the most common type, clear cell carcinoma (CCC) of the ovary is notoriously the most challenging to treat and exhibits very low response rates to standard therapies. However, efforts to understand CCC and develop new therapeutic approaches to this subtype have been limited because it represents a minority of ovarian cancer cases in the United States and Europe. In contrast, CCC accounts for more than 30% of all ovarian cancer in Japan. To improve the survival of patients with ovarian CCC, a deeper understanding of the molecular features of available model systems is needed. Our goal is to characterize a panel of CCC cell lines genomically and functionally and identify those that can serve as tractable model systems for future ***in vivo*** drug discovery studies.

METHOD: First, we characterize 9 CCC cell lines (ES-2, TOV21G, OVTOKO, OVMANA, OCI-C5x, JHOC-5, JHOC-7, JHOC-9, and OVISE) with whole exome sequence and proteomics approaches (reverse phase protein array; RPPA). Secondly, we performed ***in vitro*** assays, including soft-agar colony formation assays and MTT assays using standard chemotherapies. Thirdly, to test ***in vivo*** tumorigenic potential, by injecting 5 million cells of luciferized CCC lines in NSG female mice using both the subcutaneous route and the intraperitoneal route. Imaging was performed weekly using the In Vivo Imaging System.

RESULTS: Among the 9 CCC lines, seven (TOV21G, OVTOKO, OVMANA, OCI-C5x, JHOC-5, JHOC-9, and OVISE) harbor ***ARID1A*** mutation, which is the most prevalent mutation in CCC (50%). The second common mutation in CCC (40%), ***PIK3CA*** mutation, and was detected in 4 cells (TOV21G, OVMANA, OCI-C5x, and OVISE). The ES-2 line has ***TP53*** and ***BRAF*** mutation and its genomic profile is inconsistent with CCC. A ***BRCA2*** mutation was found in OVTOKO and JHOC-5, finding not previously reported.

Principal component analysis of RPPA data showed distinct clusters between the 9 CCC lines and the 6 HGSC lines. Interestingly, we also observed two distinct clusters within the CCC lines. Consistent with our genomic analysis, ES-2 correlated with HGSC lines based on RPPA data.

In our ***in vitro*** drug studies, OVTOKO, OCI-C5x, and OVISE exhibited a Carboplatin/Paclitaxel resistance phenotype. In xenograft study, 4 cell lines (ES-2, TOV21G, OVTOKO, and OCI-C5x) formed tumor within a month, suggesting they are useful tools for ***in vivo*** studies. In contrast, OVMANA, JHOC-7, JHOC-9, and OVISE take more than 100 days to form the tumors. Soft-agar colony formation results correlated with how long it took to develop xenograft tumor in each line.

CONCLUSION: Our genomic studies identified aberrations in CCC lines not previously described. The proteomics data revealed two clusters within the CCC lines that might represent functionally distinct groups. Finally, we identified four cell lines (ES-2, TOV21G, OVTOKO, and OCI-C5x) that readily form tumors in mice and could be used for future ***in vivo*** drug studies. However, ES-2 appears to cluster more closely with HGSC and may not represent the CCC histotype.

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CARBONIC ANHYDRASE 9 AND TUMOR HYPOXIA ARE ASSOCIATED WITH RECURRENCE AND RESISTANCE IN BRCA1-EXPRESSING HGSOC

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Tumor hypoxia contributes to poor outcome in multiple cancer types, in part by influencing the expression of DNA repair enzymes. The objective of this study was to examine the possible relationship between Carbonic Anhydrase 9 (CA-IX, an indicator of tumor hypoxia), BRCA1 expression (a potential indicator of competent DNA repair), and patient outcome in epithelial ovarian cancer. BRCA1 and CA-IX were evaluated by immunohistochemistry in a tissue microarray of 167 epithelial ovarian cancer (EOC) patient samples, and their respective expression correlated to other biological parameters in all patients. The correlations between BRCA1 and CA-IX expression, and clinical outcome were determined in the High Grade Serous Ovarian Carcinoma (HGSOC) samples (N = 101) on the array using Spearman's correlation and log-rank (Mantel-Cox) tests. Elevated expression of BRCA1 strongly correlated with shorter intervals for disease recurrence and mortality (median time to recurrence = 1.05 yrs vs 1.94 yrs, P <0.001; median time to mortality: 2.01 yrs vs 4.72 yrs, P <0.001). There was a significant positive correlation between CA-IX and BRCA1 expression (Spearman's r = 0.486, P < 0.001). CA-IX expression was significantly associated with recurrence-free survival (restricted to first 1500 days, P <0.01). When patients were stratified by BRCA1 expression, co-expression of CA-IX and BRCA1 correlated with the shortest recurrence-free interval (P <0.05). In complementary *in vitro* studies, exposure of multiple BRCA1-wild-type cell lines to hypoxia (1% O_2, mimicking general tumor hypoxia) had only a minimal effect on BRCA1 expression. Additionally, in MTT viability assays, application of the hypoxia-activated pro-drug TH-302 preferentially killed cells in 1% O_2 compared to standard conditions (21% O_2). In contrast, the PARP inhibitor olaparib was 2-5 times less effective in hypoxic conditions than in standard growth conditions, suggesting that tumor hypoxia promotes a more resistant phenotype.

CONCLUSION: The co-expression of CA-IX and BRCA1 may be a useful prognostic indicator for rapid recurrence in high grade serous ovarian cancer. Dissecting the mechanisms regulating therapeutic resistance in hypoxic ovarian cancer cells could lead to more effective treatment options for HGSOC patients.

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DECIPHERING THE ROLE OF ESTROGEN RECEPTOR ALPHA IN OVARIAN CANCER

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Ovarian cancer is the leading cause of death among gynecologic malignancies. Despite years of research, the 5-year survival rate for ovarian cancer patients has remained poor and the standard of care is largely the same as it was decades ago. Epidemiological evidence and previous studies have suggested a role for steroid hormones in the pathogenesis of ovarian cancer, however, the role of steroid hormones in ovarian cancer remains understudied. We hypothesized that Estrogen Receptor α (ERα) is transcriptionally active and drives a transcriptional program that is sufficient to promote ovarian cancer cell proliferation and survival in a subset of ER+ ovarian cancers. Preliminary data generated in our laboratory has suggested that estradiol (E2) treatment increases cell proliferation in PEO1 cells, an ERα+ ovarian cancer cell line. This increase in cell proliferation can be inhibited by co-administration with the selective estrogen receptor modulator, Tamoxifen, and the selective estrogen receptor degrader, Fulvestrant. To further interrogate the mechanism of action of ERα in ovarian cancer, we performed RNA-seq on PEO1 cells treated with Vehicle, 10 nM E2, 100 nM Fulvestrant, and E2+Fulvestrant for 24 hours. We identified 659 significantly differentially expressed genes following E2 treatment. Fulvestrant inhibited the majority of E2-induced differentially expressed genes, confirming that these genes are dependent upon ERα. Gene Set Enrichment Analysis (GSEA) indicated that the Hallmark early and late estrogen responses are enriched in the E2 dataset, confirming that classical ER activity is intact in these cells. Furthermore, we identified G2/M checkpoint as positively enriched in our dataset, indicating that proliferation genes are upregulated by ERα in these cells. Additionally, we found that apoptosis was negatively enriched in our dataset, indicating that apoptosis pathway genes are suppressed by E2 treatment. To further characterize the transcriptional role of ERα in ovarian cancer cells, ChIP-seq was performed on PEO1 cells treated with Vehicle, E2, Tamoxifen, or E2+Tamoxifen for 45 minutes. E2 treatment robustly increased ER recruitment to its regulatory regions (25,717 binding sites) when compared to Vehicle (8,370 binding sites) or Tamoxifen (18,935 binding sites) alone. Motif analysis of these ERα binding sites demonstrated a significant enrichment in members of the AP-1 transcription factor family, but not in known ERα cofactors FOXA1 and GATA3. FOXA1 and GATA3 are important regulators of ERα-dependent transcription in breast cancer cells; our results may suggest that mechanisms governing ERα transcriptional activity are lineage dependent. Finally, Binding and expression target analysis (BETA) was performed in order to predict the activating and repressing function of ERα. This analysis demonstrated that ERα has significant repressive function in PEO1 cells, indicating that ER may be acting as a strong transcriptional repressor in ovarian cancer cells. Future investigation in this project will center on the role of AP-1 in mediating ERα-dependent transcription. Additionally, we will use CRISPR/Cas9 screening to determine genetic sensitizers to ER antagonists. We believe that these studies will provide additional drug targets that may suggest a combinatorial therapeutic approach in conjunction with endocrine therapies for the treatment of ovarian cancer.

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BUILDING AN ORGANOID-BASED MODEL FOR OVARIAN CANCER

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Over the last few years several studies led to a significant change in the field of ovarian cancer and it is currently believed that the fallopian tube and not the ovary surface epithelium (OSE) is the main origin of high-grade serous ovarian cancer (HG-SOC). Nevertheless, due to the lack of unique markers and adequate model systems the relative contribution of each tissue is not yet clear and the notion that OSE has a role in HG-SOC development was not cast aside altogether. In this work we have established novel organoid systems derived from both mouse OSE and oviduct (Ovi, the equivalent of human fallopian tube). These systems recapitulate their tissue of origin and demonstrate differences in medium requirements as well as gene expression. To establish comparable tumor progression models for both OSE and Ovi we used CRISPR-Cas9 technique and targeted commonly mutated genes in ovarian cancer (Trp53, Brca1, Nf1 and Pten). Thus, we were able to establish clones with different combinations of mutations. Histological, metaphase spread and gene expression analysis of the mutated organoid clones from both OSE and Ovi demonstrated different degrees of deviation from their wild type counterpart. This deviation became more evident as the amount of introduced mutations increased. Preliminary transplantation experiments showed that only triple mutants gave rise to tumors (Trp53, Brca1 and Pten or Trp53, Brca1 and Nf1). Moreover, Ovi mutant clones were more prone to grow into tumors in comparison to OSE as no tumors have been detected in mice transplanted with the OSE equivalent clones so far. Taken together, in this study we present the first comparable Ovi/OSE research platform that enables addressing questions related to origin and early stages of HG-SOC development.

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TARGETING WNT SIGNALING TO OVERCOME PARP INHIBITOR RESISTANCE

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PURPOSE: Epithelial ovarian cancer (EOC) has one of the highest death to incidence ratios of any cancer. High grade serous ovarian cancer (HGSOC) is the most common EOC and over 85% of HGSOCs are diagnosed at a late stage (III/IV). While about 80% of all HGSOC patients will respond to first line treatments (debulking surgeries and platinum-based therapies), the majority of patients will relapse. Poly(ADP)-ribose polymerase inhibitors (PARPi) are a promising treatment for recurring chemosensitive HGSOC. However, recurrence with resistance to both chemotherapies and PARPi is common, revealing a clinical need to understand the mechanisms underlying PARPi resistance. Canonical Wnt signaling is known to play a role in the tumorigenesis and chemoresistance of many cancers, including ovarian. Non-canonical Wnt signaling is able to inhibit beta-catenin dependent TCF/LEF transcription and loss of non-canonical Wnt signaling conveys a poor prognosis in HGSOC. Therefore, we sought to determine if aberrant canonical Wnt signaling plays a role in PARPi response/resistance in HGSOC.

METHODS: RNA sequencing was performed on PEO1 cells sensitive and resistant to a PARPi, olaparib, to determine differential expression of signaling pathways and transcription factors. This was validated with TOP/FOP-FLASH reporter assays and qPCR of genes associated with canonical Wnt signaling. Colony formation assays were performed to determine the effects of overexpressing a canonical Wnt ligand, Wnt3a, in sensitive cells treated with olaparib and to determine cell response to a Wnt inhibitor (pyrvinium pamoate; Pyr. Pam.). Levels of apoptosis after treatments with PARPi and/or Pyr. Pam. was measured with Annexin V/PI assays. DNA damage repair was assessed utilizing γH2Ax resolution and two-plasmid functional DNA repair assays. The effects of olaparib, Pyr. Pam. and combination treatments were assessed *in vivo* with a xenograft mouse model (intraperitoneal injection of PEO1-Wnt3a cells) and on *ex vivo* cultures of primary chemonaive tumors.

RESULTS: PARPi resistant cells had a significant enrichment of Wnt signaling and the TCF3/LEF transcription factors as compared to the sensitive cell lines. This also correlated with an increase in mRNA of canonical Wnt signaling activators, including the ligand WNT3A, and a decrease of Wnt repressors (SFRP1, WNT5A) in the resistant cells. Sensitive cells with Wnt3a overexpressed developed an increased resistance to olaparib. Treating PARPi resistant cells with Pyr. Pam. significantly decreased cell viability, suggesting a dependence on Wnt signaling. Combination treatment of olaparib and Pyr. Pam. resulted in a synergistic increase in apoptosis when compared to either treatment alone. PARPi resistant cells had a significant increase in the rate of γH2Ax resolution and higher DNA repair activity compared to sensitive cells. Pyr. Pam. exposure attenuated the DNA damage repair activity. In a xenograft mouse model combination olaparib and Pyr. Pam. resulted in significantly decreased tumor growth, tumor weight, and tumor nodule number as compared to control. In a primary HGSOC tumor *ex vivo* model, Pyr. Pam. significantly inhibited proliferation compared to control treatment.

CONCLUSION: Our data demonstrate that HGSOC cells upregulate canonical Wnt signaling to promote resistance to PARPi. Wnt inhibitors are a promising therapeutic approach for patients who develop PARPi resistance.

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OVARIAN CANCER CELLS INDUCE A FEEDBACK RESPONSE TO PLATINUM-BASED CHEMOTHERAPY BY ACTIVATING AN ONCOGENIC RSK-EPHA2-GPRC5A SIGNALING SWITCH

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Despite increased understanding of chemotherapy resistance, the effects of the chemotherapy itself on the oncogenic cancer cell properties and adaptive resistance mechanisms of the therapy-escaping metastatic cancer cells remain largely undefined. In this study, we show that the platinum-based chemotherapeutic drugs cisplatin and carboplatin induce a switch from the canonical EphA2 receptor tyrosine kinase activity to the stemness and invasion-associated, ligand independent signaling via EphA2 S897 phosphorylation. In patient-derived high grade serous ovarian cancer (HGSOC) cells and established ovarian cancer cell lines, the chemotherapy treatment led to a significant increase in the ratio between S897 and Y588 phosphorylation of EphA2 in conjunction with GPRC5A protein upregulation and beta1-integrin activation. Notably, high protein levels of GPRC5A were significantly associated to poor survival in patients with metastatic HGSOC, revealing a previously unknown association of this EphA2 interacting receptor with the clinical HGSOC outcome. Moreover, pharmacological inhibition of RSK serine-threonine kinases blocked both the EphA2 S897 phosphorylation and the GPRC5A induction coincident with markedly increased cisplatin and carboplatin sensitivity of the HGSOC cells ex vivo. These results identify a novel mechanism of chemotherapy-induced adaptive resistance via the RSK-EphA2-GPRC5A oncogenic signaling axis in HGSOC.

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PAN-CANCER TRANSCRIPTION FACTOR ANALYSES IDENTIFY ZNF217 AS A NOVEL DRIVER IN HGSOC

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Ovarian cancer is a rare but highly lethal gynecologic malignancy, causing around 14,000 deaths annually in the US. The current standard of care for ovarian cancer includes a combination of surgical cytoreduction and platinum-based chemotherapy. Despite these aggressive treatments, most patients suffer from fatal relapses that indicate an urgent need for novel targeted therapies. Master Transcription Factors (MTFs) are transcriptional regulators responsible for cellular identity, and are associated with cell type specific regions of active chromatin termed super-enhancers (SEs). In cancer, MTFs are drivers of tumor development across a variety of organs, and MTF interfering therapies are one of the most successful therapies available today. To further identify tumor-specific MTFs, we have developed the 'Cancer Core Transcription factor Specificity (CaCTS)' algorithm and prioritized MTFs from a pan-cancer cohort of 10,000 tumors profiled by RNA-sequencing. Results demonstrated that many tumor MTFs derived from CaCTS were specific to its normal tissue-type, validating the algorithm's ability to identify factors involved in cellular identity. On the other hand, a set of highly expressed TFs were frequently observed in multiple cancer types, indicating two distinct classes of MTFs: lineage-specific MTFs and pan-cancer MTFs.

In High-Grade Serous Ovarian Cancer (HGSOC), CaCTS prioritized 21 TFs with lineage specific MTF traits. All 21 candidate MTFs were highly expressed in primary HGSOC specimens and aligned with HGSOC SEs identified via H3K27ac chromatin immunoprecipitation sequencing (ChIP-seq). As expected, WT1 and PAX8 were identified, transcription factors previously implicated to be essential in HGSOC lineage specificity. Our algorithm also identified ZNF217, a novel candidate MTF in HGSOC. ZNF217 plays a key role in breast cancer, a tumor type that shares many epidemiological, hormonal and genetic risk factors with HGSOC. Mining of a publicly available gene knockout experiment in 13 HGSOC cell lines revealed ZNF217 to have similar levels of gene essentiality as PAX8. We are currently examining the role of ZNF217 as a MTF in HGSOC by determining its target genes, binding partners, and cellular consequences of siRNA-mediated knockdown. We expect ZNF217 to occupy SEs that drive expression of genes critical for cell identity, co-occupy SEs with other candidate MTFs, and depletion of ZNF217 to result in loss of cell state. Understanding the mechanisms of transcriptional control in HGSOC will ultimately help to identify clinically relevant drivers which can serve as novel therapeutic targets for this devastating disease.

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CTDNA PROFILING TO PREDICT PROGNOSIS AND OPTIMIZE TREATMENT IN HIGH-GRADE SEROUS OVARIAN CANCER

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Tumor cells leak their DNA into the blood stream, which allows detection of tumor mutations and copy number variants from circulating tumor DNA (ctDNA) from plasma samples. These variants offer a dynamic "molecular snapshot" to the changing landscape of genomic events occurring during tumor treatment and progression. Our aim is to translate ctDNA profiling into clinical benefit in patients with high-grade serous ovarian cancer (HGSOC).

We used a comprehensive cancer-specific sequencing panel of over 500 genes to identify mutations and copy-number alterations (CNA) in ctDNA from plasma. We collected 78 plasma samples in 12 patients: at pre-treatment, primary treatment, follow-up and possible progression. For each patient, we also collected tissue and ascites samples (from 1-4 different time points per patient, totally 21 samples) and a white blood cell sample for germline variant detection. From variation detected in ctDNA, we identified clinically relevant information to predict prognosis and detect actionable genomic alterations that could be used to target treatment.

After extensive filtering of non-somatic mutations, we detected high concordance between ctDNA and tumor tissue samples: 77% of the mutations detected in ctDNA were also detected in tumor tissue samples. Mean correlation between CNAs detected in plasma versus tissue was also high, 0.7.

We identified several actionable mutations and CNAs. For example, we identified ERBB2 amplification in pre-treatment ctDNA in a poor-responding patient (platinum free interval (PFI) 5 months). The HER2 over-expression was validated in interval tumor tissue sample with immunohistochemistry and in-situ hybridization. Based on these results, the patient was treated with trastuzumab combined with reduce-dose carboplatin and dose-dense paclitaxel during disease progression, which yielded promising clinical response. In two other patients, mTOR pathway activation was predicted based on mutations detected in ctDNA. In both patients, the activation was validated with immunohistochemistry. These patients could benefit from mTOR inhibitors in case of disease progression. These identified clinically relevant variants illustrate the clinical value of ctDNA in the treatment of HGSOC patients.

Overall, patients with longer PFIs showed fast response to chemotherapy: ctDNA level was considerably reduced and mutational composition changed after first cycles of chemotherapy. Contrary, the poor-responding patients with PFI less than 12 months showed failure to drop ctDNA level after start of chemotherapy, smaller changes in mutational composition during primary treatment and higher number of detected mutations.

The early prognosis prediction in combination with identification of clinically relevant variants can allow window of opportunity to treat poor-prognosis patients even before relapse. Additionally, ctDNA allows detection of changes in mutational composition during treatment that can reveal subclonal selection which cannot be covered by single biopsies.

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IDENTIFICATION OF A NOVEL CANDIDATE BIOMARKER FOR DIAGNOSIS AND THERAPEUTIC OVARIAN CANCER TARGET

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Ovarian cancer is the most fatal gynecological cancer with high mortality. It still has no reliable diagnostic marker and effective treatment due to its high drug resistance and high recurrence. To develop a novel biomarker for diagnosis and therapeutic target of intractable ovarian cancer, we selected for an upregulated gene in serous ovarian tumors compared with normal ovary counterparts by microarray analysis and confirmed its elevated mRNA expression in ovarian tumors by RT-PCR. Consistent with its high mRNA level, tumor-specific preferential high expression of candidate protein was detected in the cytoplasm of the ovarian tumor tissues by immunohistochemistry. To our surprise, indirect immunofluorescence staining revealed an unexpected cytoplasmic subcellular localization of a candidate protein. Furthermore, forced expression of a candidate gene in cultured cells with no detectable level showed a marked inhibition of cell proliferation. To address these unexpected findings, we set out to identify a candidate interacting protein by yeast 2-hybrid screening to explore a novel function and its underlying mechanism. Among them, one of cell death associated protein was identified as a novel interacting partner of a candidate protein and their physical interaction in mammalian cells was confirmed by co-immunoprecipitation assay and confocal microscopy. Further domain mapping analysis should reveal critical domains for their interaction and would provide fundamental and pivotal information for a novel function and an underlying molecular mechanism of a candidate gene. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018R1D1A1B07047640).

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PAX8 PLAYS AN ESSENTIAL ROLE IN HIGH GRADE SEROUS OVARIAN CANCER VIA ACTIVATION OF MUTANT P53 AND MISLOCALIZED P21

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High grade serous carcinoma (HGSC) is the most common subtype of ovarian cancer, and a very deadly disease, mostly due to lack of early detection methods. Wide spread disease at diagnosis has delayed the understanding that it often originates from the fallopian tube. This novel understanding has led to increased interest in fallopian tube developmental lineage markers, such as PAX8. In the adult fallopian tube, PAX8 is expressed in the fallopian tube secretory epithelial cell (FTSEC) and its expression is maintained through the process of FTSEC transformation Serous Tubal Intraepithelial Carcinoma (STIC) and to HGSC. We show that PAX8 has an essential pro-proliferative and anti-apoptotic role in HGSC. This role is mediated through direct transcriptional activation of mutated *TP53*, which often carries missense mutations that potentially lead to gain of function (GOF) oncogenic activities. Surprisingly, mutant p53 binds the p21 promoter and transcriptionally activates the expression of p21, which localizes to the cytoplasm of cells where it plays a non-canonical, pro-proliferative role. While in normal fallopian tube p21 is nuclear as expected, and STIC shows a mix of nuclear and cytoplasmic staining, an analysis of p21 staining in EOC shows that cytoplasmic p21 expression correlates with bad prognostic factors. Together, our findings illustrate that in HGSC *TP53* mutations and abnormal p21 localization subvert a normal developmental pathway into a driver of tumor progression.

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CCNE1 AND *BRD4* EXPRESSION AND PLATINUM RESISTANCE IN HIGH-GRADE SEROUS OVARIAN CANCERS

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BACKGROUND: Epithelial ovarian cancer (EOC) is the deadliest gynecologic cancer and the fifth leading cause of cancer deaths among women in the United States. An even poorer prognostic type of EOC is ***BRCA*** wild-type ***CCNE1*** (cyclin E) amplified/gain tumors. This subtype of EOC is characterized by unchecked replication, high levels of genomic instability, and replicative stress. *CCNE1* amplified EOC is mutually exclusive to ***BRCA*** mutated EOC in part because ***CCNE1*** amplified cells require functional BRCA for survival. Bromodomain and extraterminal 4 (BRD4) amplified ovarian cancer is a recently discovered subtype of ***BRCA*** wild-type EOC, and is also associated with poor outcomes.

OBJECTIVE: To determine if mRNA and immunohistochemical (IHC) profiles of ***CCNE1*** and ***BRD4*** are associated with platinum sensitivity in serous ovarian cancers.

METHODS: Copy number analysis data was extracted from The Cancer Genome Atlas (TCGA) for high-grade serous ovarian tumors with ***BRD4*** and ***CCNE1*** (cyclin E) amplification. (www.cbioportal.org). Immunostaining for cyclin E and BRD4 was performed in 130 serous ovarian tumors on a tissue microarray (TMA) IHC in 130 clinically annotated formalin-fixed paraffin-embedded serous tumors from Vanderbilt University Medical Center (VUMC). Staining intensity (1: weak; 2: moderate; 3: strong) and percent of positive nuclei (0-100) were multiplied to yield an H score for NR4A1 expression. Pearson correlation coefficients were determined for mRNA expression of cyclin E and BRD4 in 307 TCGA tumors (RSEM V2 data extracted from the Broad Firehose database) and protein expression of cyclin E and BRD4 in 130 serous ovarian tumors. CCNE1 and BRD4 expression in relation to platinum sensitivity were evaluated using the Mann-Whitney t test.

RESULTS: The Cancer Genome Atlas (TCGA) demonstrated about 20% of high-grade serous EOC harbor amplifications in *CCNE1* and *BRD4*. *BRD4* amplification overlaps with *CCNE1* amplification in 26/57 (46%) of high-grade serous EOC of the TCGA. Immunostaining for cyclin E and BRD4 in 130 serous ovarian tumors on a tissue microarray yielded intermediate-to-high staining of CCNE1 in 52.1% of tumors and intermediate-to-high staining of BRD4 in 71.6% of tumors. Protein expression by IHC between CCNE1 and BRD4 was positively correlated, r= 0.25, (p =0.005). High expression of CCNE1 was associated with platinum resistance (p = 0.023). High BRD4 expression was not associated with platinum resistance (p=0.95).

CONCLUSION: TCGA mRNA and TMA IHC expression analysis suggest that a subset of serous ovarian tumors have high levels of CCNE1 and BRD4 expression. High CCNE1 expression is associated with poor prognosis and platinum resistance. High BRD4 expression is not associated with platinum resistance in this cohort. Future studies are warranted to evaluate the subset of tumors with elevated expression of both CCNE1 and BRD4 in relation to platinum resistance and clinical outcomes.

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GENOMIC CHARACTERIZATION OF ADULT-TYPE GRANULOSA CELL TUMORS: IMPLICATIONS FOR PATHOGENESIS AND TREATMENT OF RECURRENT DISEASE

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BACKGROUND: Adult granulosa cell tumors (AGCT) represent 3-5% of all ovarian cancers. These tumors are characterized by their slow growth and usually occur in postmenopausal women with a median age of diagnosis of 50 to 54 years. The majority of patients are diagnosed as stage I and are treated with surgery to remove their ovaries and uterus. Although these treatments are effective at first, one third of patients relapse leading to mortality in 50-80% of relapsed patients. The best course of treatment for patients with recurrent advanced stage disease is optimal debulking surgery. Currently, there are no effective treatments available for patients where surgery is not an option. Our research team previously discovered a somatic missense mutation (c.402C>G; pC134W) in the transcription factor Forkhead box L2 (***FOXL2***) in 97% of AGCTs. We also discovered frequent activating telomerase reverse transcriptase (***TERT***) promoter mutations in AGCT. As the ***FOXL2*** C134W mutation is present in essentially all AGCTs and telomerase reactivation is required for tumorigenesis, it is likely that additional mutations are responsible for the variability in clinical behaviour. This study aims to describe the mutational landscape of AGCT to further refine our understanding of the frequent recurrence of this disease.

METHODS: Using whole genome sequencing (WGS), we characterized the genomes of ten AGCTs and their matched normal blood. We observed that AGCTs have a low mutation burden and the majority of mutations are single nucleotides variants. We have collected 516 formalin-fixed paraffin-embedded AGCT specimens including primary, recurrent and metastatic tumors from seven international centres for validation of our WGS results. Allelic discrimination assays were used for hotspots mutations, in addition to targeted sequencing of 39 genes of interest using a custom amplicon-based panel in our extension cohort.

RESULTS: Of the 39 genes analyzed, the third most commonly mutated gene (***FOXL2*** and ***TERT*** being the first and second most common) in our preliminary analysis of 88 cases was lysine (K)-specific methyltransferase 2D (***KMT2D*** or ***MLL2***). We identified various missense and nonsense mutations in this gene in 16 of 88 AGCTs (18%) analyzed thus far. ***KMT2D*** is a histone methyltransferase that targets histone H3 lysine 4 (H3K4), a methylation activation mark, and has an essential role in transcriptional regulation. Using an allelic discrimination assay, we identified a known hotspot mutation (c.49G>A;p.E17K) in v-akt murine thymoma viral oncogene 1 (***AKT1***) in 2 of 67 (3%) AGCT patients, one of which the mutation was present in all three recurrent specimens from the same patient. ***AKT1*** E17K mutation has been reported in multiple cancers such as breast, colorectal and high grade serous ovarian cancer at a low prevalence. A recent clinical trial of AKT inhibition in solid tumors with ***AKT1*** mutations included one recurrent AGCT patient and showed significant tumor regression in one metastatic site.

CONCLUSION: ***AKT1*** E17K mutations are present in AGCT at a low prevalence and could represent a therapeutic target for patients with recurrent advanced stage disease harbouring this mutation.

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CTDNA RESPONSE TO THE PARP INHIBITOR RUCAPARIB PREDICTS PROGRESSION-FREE SURVIVAL AND BEST OVERALL RESPONSE ON THE ARIEL2 TRIAL

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BACKGROUND: High grade serous ovarian carcinoma (HGSOC) is characterized by ubiquitous ***TP53*** mutation and has the highest genomic complexity of all epithelial neoplasms. Sensitivity to PARP inhibitor therapy is strongly associated with homologous recombination deficiency (HRD). Genomic biomarkers of HRD such as genome-wide loss of heterozygosity (LOH) scores predict HRD and response to rucaparib. We hypothesized that functional testing of response during PARP inhibitor treatment using changes in circulating tumour DNA (ctDNA) could improve prediction of patient outcome. We tested whether the change in ctDNA ***TP53*** mutant allele fraction (MAF) after one cycle of rucaparib treatment was predictive of progression free survival (PFS) and response in patients from the phase 2 ARIEL2 trial in women with platinum-sensitive recurrent high grade ovarian cancer (NCT01891344).

METHODS: We analyzed serial plasma samples (n = 636) from 142 HGSOC patients during screening, on day 1 of each treatment cycle, and at the end of rucaparib treatment. Targeted amplicon deep sequencing (TADS) of ***TP53*** was performed on DNA extracted from plasma (median depth 6916×). Somatic ***TP53*** mutation and loss of heterozygosity score (LOH) were available from archival and biopsy specimens. Statistical analyses were pre-specified and ctDNA analysis was carried out blinded to visit and response data. ***TP53*** MAF changes after one cycle of treatment were compared with PFS and best overall response assessed by RECIST v1.1 and GCIG CA-125 criteria. Optimal cut points for ctDNA response were determined using a cross-validation analysis. In cases with >1 ***TP53*** mutation, response assessment was performed using the mutation with highest MAF.

RESULTS: We detected ***TP53*** mutations in plasma from 134 patients; all cases were concordant between tumour and plasma except for one patient (present in plasma but not tumour). In 18 patients (13%), 2 or more ***TP53*** mutations were detected in ctDNA. The median ***TP53*** MAF prior to cycle 1 was 2.6% (IQR 0.3–8.6). Reduction of >70% of ***TP53*** MAF in ctDNA between cycle 1 and 2 was significantly predictive of improved PFS (n = 97; HR = 0.53, 95% CI 0.34–0.85, p = 0.008, median 273 vs 158 days, sensitivity 76%, specificity 62%) and best overall response (n = 97; OR = 7.04, 95% CI 2.69–21.06, ***p*** < 0.001). Combining ctDNA and LOH scores did not improve prediction of response.

CONCLUSIONS

Response measured by >70% fall in ***TP53*** ctDNA between pre-cycle 1 and pre-cycle 2 of rucaparib therapy was significantly associated with best overall response and improved PFS. Similar findings were observed in a retrospective study of recurrent HGSOC treated with standard of care chemotherapy. The pathological or genomic factors causing multiple ***TP53*** mutations in ctDNA are unknown.

The association between early fall in ctDNA and validated RECIST and CA-125 response markers provides strong evidence that ctDNA may have utility for detecting early response to targeted therapy. Further analyses in randomized studies should be performed to confirm that ctDNA response has strong predictive value.

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STAT3 PROMOTES OVARIAN CANCER GROWTH AND DRUG RESISTANCE BY MODULATING THE ENERGY METABOLISM

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Signal Transducers and Activators of Transcription 3 (STAT3) is a transcription factor that is known to play a key role in cancer progression. In ovarian cancer, STAT3 overexpression leads to increased cancer cell proliferation and confers resistance to chemotherapy-induced apoptosis in epithelial malignancies. It is constitutively activated in patient-derived ovarian cancer cells and a predictor of poor prognosis. Apart from its function as a transcription factor, recently STAT3 has been shown to translocate to mitochondria facilitated by phosphorylation at S727 and modulate mitochondrial function to promote carcinogenesis.
Our study aimed to investigate if STAT3 overexpression can modulate cellular metabolism and promote the growth of ovarian cancer cells. We generated stable clones overexpressing STAT3 in A2780 ovarian cancer cells, along with empty vector clones. Ectopic expression of STAT3 in A2780 resulted in increased proliferation, colony formation ability and chemoresistance in vitro and led to large and aggressive ovarian tumors compared to parental and vector controls in xenograft mouse model. STAT3 overexpressing clones exhibited higher mitochondrial respiration and glycolysis placing them in the 'metabolically active' phenotype compared to parental and vector clones (metabolically less active phenotype). A selective inhibitor of STAT3, Stattic, inhibited both nuclear and mitochondrial STAT3 and also attenuated the STAT3 mediated growth of over-expressing clones both in vitro and in vivo. Stattic treatment also reversed the STAT3-mediated chemoresistance. In contrast, a selective inhibitor of STAT3-Y705, Cryptotanshinone was relatively less effective. Also, Stattic treatments reversed the 'metabolically active' state of STAT3 overexpressing clones to a 'lower metabolic state,' as the control cells. Stattic also inhibited the cell proliferation and modulated bioenergetic phenotype of other ovarian cancer cells lines (PEO4, C200, and OVCAR3) that display a metabolically active phenotype suggesting STAT3 plays a vital role in attaining a metabolically active phenotype by cancer cells. Although, an increase in mitochondrial function was observed in overexpressing A2780 clones as evident from enhanced oxidative phosphorylation, there is no change in the mitochondrial mass or number in overexpressing clones compared to parental A2780 and vector clones indicating the critical role of STAT3 in mitochondrial functions rather than mitochondrial biogenesis in ovarian cancer cells. Further, evaluation of expression and function of mitochondrial STAT3 in ovarian cancers is warranted. Overall, STAT3 can induce metabolic changes in ovarian cancer cells by facilitating mitochondrial function, maybe as a survival mechanism and enhances the cellular fitness of the ovarian cancer cell resulting in increased oncogenic abilities.

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IDENTIFICATION OF NOVEL CHROMOSOMAL REARRANGEMENTS IN HIGH-GRADE SEROUS OVARIAN CANCER

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High-grade serous ovarian cancer (HGS-OVCA) is the most common type of ovarian cancer. HGS-OVCA genomes are usually very complex and unstable. Many studies have addressed cytogenetic changes, and with the advent of high-throughput sequencing, complex chromosomal rearrangements. However, little is known about how gene fusions, potentially causing aberrant transcripts and protein products drive tumorigenesis, cell growth and/or metastasis in HGS-OVCA. Patients with the same histological or morphological phenotype of cancer have tumors with diverse genetic backgrounds or molecular phenotypes. There is thus an urgent need to better stratify patients and their tumors and to understand the chemotherapy and other treatment responses at the molecular level.

The purpose of this study is to find and charaterize novel recurrent chromosomal rearrangements from HGS-OVCA patient samples. We aim to identify rearrangement hotspots from genomic data. However, not only do we need to identify, characterize and stratify the genomic alterations, but also to find those changes with potentially tumorigenic functions at the RNA and protein levels.

We utilise datasets of The Cancer Genome Atlas (https://cancergenome.nih.gov/) and HERCULES Project (http://www.project-hercules.eu/) to identify rearrangement-prone chromosomal regions (putative “breakpoint hotspots”) and complex structural aberrations and variations. We concentrate on exonic aberrations seen in both datasets in at least 2 patients. The findings will be validated using two HGS-OVCA fresh-frozen tumor sample collections from Helsinki and Turku University Hospitals. Clinical data and homologous recombination (HR) score of the tumor samples (HR proficient or HR deficient; Tumiati et al. 2018) will also be analyzed.

Preliminary results of this work will be presented. We believe that combining sequencing and clinical data with state-of-the-art molecular level analyses and functional tests will deepen our understanding of HGS-OVCA biology and help to develop new anti-cancer treatment options for this “silent killer”.

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A CLIA-CERTIFIED HIGH-THROUGHPUT DRUG SCREENING PLATFORM FOR OVARIAN CANCER TO INFORM PERSONALIZED CANCER CARE AND DISCOVER NOVEL COMBINATIONS

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BACKGROUND: Metastatic disease in ovarian cancer is difficult to treat and patients often exhaust standard-of-care regimens. To gain a better understanding of potential treatments, genomic data can be used. However, the interpretation of the genomic data for ovarian cancer is challenging given complex mutation patterns and the absence of recurrent druggable alterations beyond DNA repair deficiency. Here we present the first CLIA certified high-throughput functional assay employing organoid cultures derived from primary patient specimens to directly aid oncologists for personalized treatment selection in combination with genomic data (P.A.R.I.S® Assay, SEngine Precision Medicine, Seattle, WA).

EXPERIMENTAL PROCEDURES: Organoids are exposed to a library of 123 clinically relevant drugs. The library was developed to include chemotherapies and FDA approved drugs currently used in the clinic, as well as promising drugs undergoing clinical trials. Compounds are evaluated at a multi-dose response curve and ranked by SPM™ score, which weighs both the sensitivity (degree of cell death) and specificity, which compares the response of the patient’s tumor cells to the drug relative to all prior patients. In addition to single agent screens, optimal combinations can also be tested. The results are further integrated with genomic data when available, and drug-responses are reported to the clinician. SEngine has performed >200 drug screens across >20 different tumor types and established high reproducibility of the high-throughput platform. Ovarian samples were derived from either ascites or biopsies.

RESULTS: Of the 7 samples directly derived from patients, 1 was from ascites, 4 were from biopsies or surgical excisions, and 2 had both. In addition, 13 cell lines were screened with the SEngine drug library. While every patient exhibited a unique pattern of response consistent with the heterogeneity of the complex genetic landscape of ovarian cancer, sensitivities for certain drugs such as HDAC, PI3K and tyrosine-kinase inhibitors were frequently found. Several n=1 cases will be presented to highlight correlations with retrospective clinical responses as well as with genomic alterations. These results highlight how genomic data and functional testing can be combined to optimize personalized cancer care.

To identify optimal drug combinations with PARP inhibitors, we have performed an unbiased screen where patient derived ovarian cancer cell lines were challenged with SEngine drug library in the presence of rucaparib. The screen indicated multiple drugs, such as bromodomain, BCL2 and cyclin dependent kinase (CDK) inhibitors as well as dasatinib as potential sensitizers to rucaparib. Confirmation of these results in additional patient derived organoids and PDX models are in progress.

IMPACT: We developed a robust ex vivo screening platform to objectively quantify patient specific sensitivity to a panel of more than 123 oncology drugs and potential novel combinations. SEngine is compiling a registry capturing clinical data, outcome following the P.A.R.I.S® test, and genomic data. Combining the power of high-throughput technology and organoid isolation with genomic data will enable the rapid selection of optimal individualized therapies as single agents or in combination and guide design of future clinical trials.

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EVIDENCE FOR APOBEC MUTAGENESIS IN CLEAR CELL OVARIAN CARCINOMA

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Clear cell ovarian carcinoma (CCOC) accounts for 1-12% of ovarian epithelial cancers and, notwithstanding early diagnosis, often has poor clinical outcomes. CCOC genomic sequences show strong cytosine to thymine (C-to-T) transition mutation biases (Wang et al., 2017, Nature Genetics; Maru et al., 2017, Gynecologic Oncology; Shibuya et al., 2018, Genes Chromosomes Cancer). Many of these mutations occur in APOBEC signature motifs (5'-TCA or TCT), implicating at least one of the nine APOBEC DNA cytosine deaminase family members in CCOC mutation and evolution. Three of the nine family members associate with cancer mutagenesis, but only one, APOBEC3B (A3B), correlates in multiple cancer types with worse clinical outcomes including overall and metastasis-free survival.

Here, we further investigate the specific involvement of APOBEC mutagenesis in CCOC. First, we show that tumors positive for APOBEC signature mutations associate with poor clinical outcomes in comparison to those that do not. Second, we perform a comprehensive analysis of mutation signatures from new whole genome sequences and APOBEC expression profiles from tumor and normal tissues, including RTqPCR for mRNA quantification and immunohistochemistry to distinguish APOBEC protein expression in tumor cells from surrounding connective tissue and immune cells. Our results indicate a significant role for APOBEC mutagenesis in CCOC warranting further studies including evaluation of APOBEC as a prognostic biomarker.

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ROLE OF LATS AND DYRK1A KINASES IN THE PATHOGENESIS AND TREATMENT OF HGSOC

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Epithelial ovarian cancer (EOC) is the most lethal gynecological malignancy, with 75% of women presenting with metastatic disease at the time of diagnosis. High Grade Serous Ovarian Carcinoma (HGSOC) is the most common and aggressive type of epithelial ovarian cancer, with 5-year survival rate of 30% for most patients. Developing of accurate experimental models of HGSOC is necessary to elucidate the disease pathogenesis and to evaluate new treatments. Our study is aimed at determining the roles of the Large Tumor Suppressor 1 and 2 kinases (LATS1/2), and their downstream effector Dual-specificity tyrosine(Y)-Regulated Protein Kinase 1A (DYRK1A), in HGSOC pathogenesis and treatment sensitivity. The Cancer Genome Atlas study revealed copy number losses of the LATS1, LATS2 and DYRK1A genes in 65%, 59% and 38% of HGSOC cases, respectively. Previous studies found that LATS1/2 and DYRK1A mediate a crosstalk between the Hippo tumor suppressor pathway and the G0/G1 cell cycle checkpoint. However, the role of these kinases in HGSOC pathogenesis is not known. We found that loss of both LATS1 and LATS2, or DYRK1A results in increased proliferation of SKOV3 ovarian cancer cells in 2D or 3D cultures, mediated in part by disruption of the DREAM complex that represses cell cycle-regulated genes. Interestingly, we observed upregulation of cyclin D1 and increased phosphorylation of retinoblastoma (RB) family proteins in SKOV3 cells depleted of LATS1/2 or DYRK1A kinases, indicative of aberrant activation of CDK4/6. Similar changes were observed upon depletion of LATS1/2 or DYRK1A using non-transformed human fallopian epithelial cells, suggesting that genetic losses of these kinases could contribute to the HGSOC pathogenesis. Furthermore, we tested whether an aberrant activation of CDK4/6 in SKOV3 cells with low levels of LATS1/2 or DYRK1A could increase their sensitivity to pharmacological CDK4/6 inhibitors such as palbociclib. Using metabolic cell proliferation assays, we found that loss of LATS1/2 or DYRK1A resulted in a significant decrease of palbociclib IC50 in SKOV3 cells. Interestingly, palbociclib treatment increased the DREAM assembly in these cells, resulting in downregulation of DREAM-regulated cell cycle factors such as cyclin A or B-Myb. Our results show that loss of LATS1/2 or DYRK1A could contribute to HGSOC pathogenesis by CDK4/6-mediated disruption of RB family function. Further studies are underway to validate our findings in vivo using orthotopic ovarian tumor xenografts in mice and a bioluminescence imaging approach. In addition, these models will be used for validation of novel therapeutic approaches for HGSOC, such as palbociclib, as well as for the mechanistic studies of the EOC pathogenesis.

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AN INVESTIGATION OF THE EFFECTS OF THE ATYPICAL PKC-ζ INHIBITOR ζ-STAT ON CLEAR CELL CARCINOMA OVARIAN CANCER PROLIFERATION AND INVASION

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Ovarian cancer is the most lethal gynecological cancer diagnosis. There is no efficient screening process for ovarian cancers and the average stage of diagnosis is stage III. The most common ovarian cancer diagnosis is epithelial ovarian carcinomas, which constitute for 85-90% of prognosis; of this percent, clear cell ovarian carcinoma (CCOC) represents 5% of incidence. CCOC presents unique pathological features and has a high reoccurrence rate after treatment. An overexpression of atypical PKCs (PKC-ζ and PKC-ι/λ) has been observed in various malignant cells lines and are linked to pathways for cellular proliferation and invasion. In this investigation, ovarian cell lines (TOV21G and ES-2) were treated with the atypical PKC-ζ inhibitor ζ-Stat and assayed to determine the effects on proliferation and cellular invasion. These assays included protein quantification, cell proliferation, and wound healing. Mouse xenograph experiments were also performed to determine the effects of ζ-Stat on TOV21G tumor growth in vivo. Our data shows that ζ-Stat decreases the proliferation of clear cell carcinoma ovarian cancer cells and decreases wound healing. Our results also show that tumor growth in athymic female mice is decreased when treated with ζ-Stat and that mouse body weight is maintained. This suggests that PKC-ζ is a novel target in carcinogenesis and inhibition of this protein decreases the rate of proliferation.

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BMI1 AND RING1A ARE INVOLVED IN H2A UBIQUITINATION AT SITES OF PLATINUM-INDUCED DAMAGE

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Ovarian cancer is the 5th leading cause of death among women with the five year survival rate for ovarian cancer patients being only 46%. The standard of care for treatment of ovarian cancer patients is debulking surgery followed by platinum-taxane based chemotherapy. However, the major obstacle in the treatment of these patients with platinum based agents is development of chemoresistance. The platinum agents, cisplatin and carboplatin damage DNA by forming adducts with adjacent guanines, a high density of which are found in the promoter CpG islands of genes. Aberrant promoter DNA hypermethylation of genes and their subsequent transcriptional repression has been associated with development of cisplatin resistance. However, the mechanism of initiation of this aberrant DNA methylation and subsequent transcriptional repression is not known. Our overall hypothesis is that platinum induced DNA damage or repair of the damage leads to recruitment of repressive proteins to sites of damage. These repressive proteins transiently repress transcription in vicinity of damage to promote repair. However, similar to our findings with enzyme induced double strand breaks, repressive proteins may be retained at some key loci causing persistent transcriptional repression and gene silencing contributing to the development of platinum resistance. Our preliminary data demonstrates that BMI1, a member of polycomb repressive complex 1, localizes to sites of damage after cisplatin treatment. We also observe mono-ubiquitination of H2AX after cisplatin treatment and we hypothesize that this ubiquitination is occurring on K119. Mono-ubiquitination of H2A/H2AX at K119 has been associated with transcriptional repression and gene silencing during development and differentiation and also during repair of ionizing radiation and enzyme induced double strand breaks. Our data suggests that knockdown of RING1A reduces the cisplatin induced H2AX ubiquitination. We are currently determining which repair pathways and/or proteins result in recruitment of BMI1, RING1A to sites of damage. Importantly, after acute cisplatin treatment we also observe a reduction in expression of candidate genes which are known to be methylated in cisplatin resistant cells. We plan to determine if RING1A mediated ubiquitination is responsible for reduction in expression of target genes after acute cisplatin treatment and their methylation in cisplatin resistant cells. Understanding the mechanism of platinum induced gene silencing will enable us to design therapies to avert the development of drug resistant ovarian cancers.

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IGF-AXIS SIGNALING DURING OVULATION IS RESPONSIBLE FOR THE STEMNESS CELL EXPANSION AND TRANSFORMATION OF FALLOPIAN TUBE FIMBRIAL EPITHELIUM

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Recent studies have unveiled the fallopian tube fimbria as the major tissue-of-origin and incessant ovulation as the main etiological risk factor of ovarian high-grade serous carcinoma (HGSC) but the mechanism of cancer initiation remains elusive. In a series of study we have found a subset of preovulatory follicular fluids (FF) contain high level of ROS that induce DNA double strand breaks as well as apoptosis to the fimbrial epithelial cells in vitro and in vivo. We also found hemoglobin in pelvis, most likely contributed by retrograde menstruation of previous cycles, reduces the ovulatory ROS level extracellularly and results in a shifting from the apoptotic to the survival fate of ROS-stressed fimbrial epithelial cells. These initiating factors in FF, although may contributes to the TP53 mutation and other early genetic hits in the genesis of HGSC, cannot fully transform the immortalized fimbrial epithelial cells, with loss of p53 and Rb.

In this study, we demonstrated that IGF axis proteins, including IGFBP2- and IGFBP6-bound IGF2 as well as the IGFBP-lytic enzyme PAPP-A are abundantly present in ovulatory FF, and disclosed their effect on the fallopian tube fimbrial epithelium. Upon engaging with glycosaminoglycans (GAG) on the membrane of fimbrial epithelial cells, PAPP-A cleaves IGFBPs and releases bioactive IGF2 in close proximity to its receptor IGF-1R. This leads to stemness activation and clonal expansion of primary and immortalized fimbrial epithelial cells, and to malignant transformation of p53/Rb- and p53/CCNE1-disrupted fimbrial secretory cells. By antibody depletion, we proved each component of this IGF axis is essential and confers the majority of the transformation/regeneration activities in FF. Two signaling pathways, IGF-1R/ AKT/mTOR and IGF-1R/AKT/NANOG, are responsible for the phenotypes. Among them, IGF-1R and NANOG were critical since blocking their expression or function lead to a complete loss of the transformation.

Given that ovulation is an acute inflammatory process inevitably damaging the ovarian surface and the fimbriae, IGF axis in FF takes a role of tissue repair and, in situations when the ROS-induced TP53/Rb mutations accumulated in stem cells, may lead to carcinogenesis of the fallopian tube fimbria.

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A FUNCTIONAL HOMOLOGOUS RECOMBINATION ASSAY PREDICTS PRIMARY CHEMOTHERAPY RESPONSE AND LONG-TERM SURVIVAL IN OVARIAN CANCER PATIENTS

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PURPOSE: Homologous recombination deficiency (HRD) correlates with platinum sensitivity in ovarian cancer patients, which clinically is the most useful predictor of sensitivity to PARPi. For this reason, there is clearly a critical need to identify homologous recombination deficiency (HRD) as early as possible. Currently, HRD is mostly diagnosed by genetic testing, which however fails to identify a large proportion of HR-deficient tumors and predict a patient's response to chemotherapy, thus we aimed to develop an ex vivo functional HRD detection test that could predict both platinum-sensitivity and patient eligibility to targeted drug treatments.

METHODS: We obtained a functional HR score by quantifying homologous recombination (HR) repair after ionizing radiation-induced DNA damage in primary ovarian cancer samples (n=32). Samples clustered in 3 categories: HR-deficient, HR-low and HR-proficient. We analysed the HR score association with platinum sensitivity and treatment response, platinum-free interval (PFI) and overall survival (OS), and compared it with other clinical parameters. In parallel, we performed whole-genome sequencing and targeted DNA-sequencing of HR genes to assess if functional HRD can be predicted by currently offered genetic screening.

RESULTS: Low HR scores predicted primary platinum sensitivity with high statistical significance (p=0.0103), associated with longer PFI (HR-deficient vs HR-proficient: 531 vs 53 days), and significantly correlated with improved OS (HR score <35 vs ≥35, hazard ratio=0.08, p=0.0116). At the genomic level, we identified a few unclear mutations in HR genes and the mutational signature associated with HRD, but, overall, genetic screening failed to predict functional HRD.

CONCLUSIONS: We developed an ex vivo assay that detects tumor functional HRD and an HR score able to predict platinum sensitivity, which holds the clinically relevant potential to become the routine companion diagnostic in the management of ovarian cancer patients.

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CBX2 IDENTIFIED AS DRIVER OF ANOIKIS ESCAPE AND DISSEMINATION IN HIGH GRADE SEROUS OVARIAN CANCER

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The vast majority of high grade serous ovarian carcinomas (HGSOC) are diagnosed at an advanced stage. Chromobox 2 (CBX2), a polycomb repressor complex subunit, plays an oncogenic role in a variety of cancers. In prostate cancer, CBX2 is a driver of metastatic progression. Little is known about the role of CBX2 in HGSOC. Our hypothesis is that CBX2 upregulation promotes advanced HGSOC by promoting a stem-like transcriptional profile and inhibiting anoikis. Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) were queried to establish the role of CBX2 in HGSOC. In vitro evaluation of CBX2 occurred in OVCAR4, PEO1, and OVCAR8 HGSOC cell lines. PolyHEMA-coated plates forced cells to grow in suspension and simulated anoikis-escape. Quantitative polymerase chain reaction and immunoblots evaluated CBX2 expression. Small hairpin RNAs (shRNAs) knocked CBX2 down for loss of function studies. To mimic HGSOC progression several culture conditions, including 2D, colony formation, and 3D, spheroid, were examined. Secreted Gaussia luciferase (gLuc) activity was utilized as an indicator of proliferation. Stemness was tested with the Aldefluor assay, measuring aldehyde dehydrogenase activity (ALDH). Using patient tumors derived from the Gynecology Tissue and Fluid Bank (GTFB) and a HGSOC tissue microarray (TMA) with matched primary, metastatic, and lymph nodes, a CBX2 expression profile was established. Student's t-test was used to define statistical significance, with a p value of < 0.05. Analysis of GEO databases established CBX2 is upregulated in HGSOC tumors compared to benign tissues. Analysis of TCGA found CBX2 expression conveyed worse disease-free survival (11.7 vs 17.6 months, Log-rank test p-value < 0.005) and overall survival (34 vs. 44.8 months, Log-rank test p-value <0.005). Examination of primary HGSOC tumors confirmed CBX2 was upregulated at the protein level in HGSOC compared to benign tissue. In vitro, OVCAR4, PEO1, and OVCAR8 cells upregulate CBX2 when grown in suspension compared to adherent conditions. CBX2 knockdown led to a significant inhibition of proliferation in 2D, 3D, and in suspension. Forced suspension promoted increased ALDH3A1 expression and ALDH activity. CBX2 knockdown led to a decrease in both ALDH3A1 expression and ALDH activity. HGSOC cells grown in suspension were found to be more chemoresistant compared cells grown under adherent conditions. Similarly, knockdown of CBX2 sensitized HGSOC cells to cisplatin. Examination of primary tissue in a TMA of matched patient samples revealed CBX2 is expressed in primary and metastatic disease. We conclude that CBX2 directly impacts proliferation and is overexpressed in HGSOC. Our work indicates that CBX2 is an important regulator of stem-ness, which could play a role in anoikis escape, HGSOC dissemination, and chemoresistance, suggesting that CBX2 may be associated with more advanced disease. This exploration expands our understanding of molecular drivers of HGSOC progression and potentially identifies a novel therapeutic target.

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SHEAR STRESS DOWNREGULATION OF MIR-199A-3P DRIVES CHEMORESISTANCE IN OVARIAN CANCER CELLS

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Ovarian cancer is leading cause of deaths among all gynecological malignancies. The presence of intrinsic and acquired drug resistance are major challenges in the treatment and clinical management of ovarian cancer. Ovarian cancer cells typically cluster into tumor spheroids and float in the ascitic fluid, which are characteristic of the aggressiveness of these tumor cells. We have recently developed a three-dimensional microfluidic platform with tumor spheroids and continuous fluidic flow to closely emulate the physiological condition in the ascites of ovarian cancer patients. Using this model, we showed for the first time that clinically relevant levels of ascitic shear stress induced drug resistance in ovarian tumor spheroids. Such chemoresistance was mediated through the increased expression of ABC-binding cassette transporter G2 and P-glycoprotein. By profiling the microRNAs (miRNAs) expression patterns under flow conditions, we identified miR-199a-3p as a critical mechanosensitive miRNA that was downregulated under shear stress through regulating the miRNA biogenesis machinery. miR-199a-3p expression was found to be inversely correlated with enhanced drug resistance properties and chemoresistant ovarian cancer sublines. Ectopic expression of miRNA-199a-3p could reverse the shear stress-induced expression of ABC-binding cassette transporter G2 and P-glycoprotein in ovarian tumor spheroids, confirming that the effect was miR-199a-3p specific. Taken together, these findings highlight the importance of shear stress-mediated decrease of miR-199a-3p in the regulation of drug response in ovarian cancer, which provide new insights in the understanding of cancer biology and potential effective treatment.

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A NOVEL TREATMENT APPROACH FOR TARGETING CYCLIN E OVER-EXPRESSING OVARIAN CANCERS

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Ovarian cancer lacks therapies that yield complete tumor regression resulting in low cure rates. Cyclin E (*CCNE1*) is an oncogenic driver that is amplified in 25% of high grade serous ovarian cancers (HGSOC). Cyclin E overexpression (CCNE1^{HIGH}) is associated with poor survival and platinum resistance in these cancers. Effective therapies for CCNE1^{HIGH} ovarian cancers yielding complete and durable responses are lacking. Aberrant expression of Cyclin E leads to unscheduled entry into S phase, premature origin firing, nucleotide depletion, and replication fork stress. The imbalances created by this abnormal progression leads to an increased reliance on cell cycle checkpoint regulators, such as WEE1 and ATR, which counter some of the untoward effects of Cyclin E overexpression. WEE1 is a dual specificity kinase that regulates cell cycle progression by inhibiting both CDK2 and CDK1, thereby inhibiting progression from G_1 to S and G_2 to M phases, respectively. ATR kinase protects the replication fork from collapse thereby inhibiting G2/M progression so DNA can repair. Additionally, mutations in another G1-S phase checkpoint regulator, *TP53*, may accentuate dependence on WEE1 and ATR by eliminating an alternative checkpoint pathway that limits CCNE1-CDK2 activity. *TP53* mutations are also ubiquitous in HGSOC, further implying the promise of WEE1i and ATRi in selective killing CCNE1^{HIGH} tumors as a rational therapeutic strategy. We tested drug effects on survival, colony formation, cell cycle and apoptosis in vitro and in patient-derived xenograft (PDX) models. Induction of Cyclin E expression in immortalized human fallopian tube secretory epithelial cells (FTSEC) upregulates pChk1, a downstream protein of ATR. Combination of WEE1i with ATRi (WEE1i-ATRi) synergistically decreases cell viability and colony formation in CCNE1^{HIGH} HGSOC cells. Selective inhibition of ATR and WEE1 with siRNA was synergistic supporting that ATR and WEE1 are critical to survival. WEE1i-ATRi dramatically increased cell apoptosis, decreased S phase cells and arrested cells at G2/M phase. Combination inhibition of WEE1 and ATR induced gH2AX and phosphorylation of RPA32, indicating increased double strand DNA break and replication stress. Also, combination inhibition of WEE1 and ATR promoted mitotic catastrophe, as indicated by increased pHH3. Finally, combination WEE1-ATRi is tolerable and results in a 4-fold increase in survival compared to standard chemotherapy or monotherapy in a *CCNE1* amplified HGSOC PDX model. In addition, sequential treatment is as effective and less toxic as concomitant WEE1i with ATRi warranting further study. Our studies developed a novel combination treatment approach for targeting Cyclin E over-expressing ovarian cancers.

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MOLECULAR CHARACTERIZATION OF MOUSE MODELS OF HIGH-GRADE SEROUS CARCINOMA ARISING IN THE OVIDUCT

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We recently developed genetically engineered mouse models (GEMMs) of oviductal high-grade serous carcinoma (HGSC) based on conditional inactivation of several different combinations of tumor suppressor genes (***Brca1, Trp53, Rb1***, and ***Nf1)*** that are recurrently mutated in human HGSCs. The histopathological features of the mouse tumors closely mimic their human tumor counterparts. In order to characterize how well the mouse tumors recapitulate the molecular characteristics of human HGSCs, targeted exome sequencing was used to analyze the 32 most commonly mutated genes in human HGSC, in 60 mouse tumors arising in the context of ***Brca1, Trp53, Rb1***, and/or ***Nf1*** inactivation. We employed the sequence data to assess DNA copy number alterations (CNAs) and single nucleotide variants (SNVs) in the mouse tumors.

Compared to 14 normal tissues and 8 oviductal tumors arising in the context of ***Apc, Pten,*** ± ***Arid1a*** inactivation, the mouse HGSCs showed a high level of genomic instability, with many widely distributed CNAs – very similar to the widespread CNAs observed in human HGSCs. Targeted exome sequencing also showed that a subset of the mouse tumors acquired alterations observed in human HGSCs, including amplification of ***cMyc***, and deletion of ***Pten.*** Variant analysis identified nonsynonymous SNVs in ***Csmd3***, ***Crebb***p, ***Pten***, ***Mettl17,*** and ***Zymynd8*** and a frameshift deletion of ***Pten***. Sanger sequencing confirmed the presence of these somatic mutations in the mouse tumors and their absence in matched normal tissues. Loss of PTEN expression was observed in those tumors that acquired somatic ***Pten*** alterations.

These data show that HGSCs arising in our GEMMs have very similar molecular characteristics to their human tumor counterparts. The somatic alterations are likely acquired during the relatively lengthy period (several months) between tumor initiation and progression to overt malignancy. These features render the models particularly well suited for studying the early phases of HGSC development and for translational applications aimed at identifying effective strategies for HGSC prevention and early detection.

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TARGETING OVARIAN CANCER STEM CELLS THROUGH THE TUMOR SUPPRESSOR DAB2IP-MEDIATED WNT SIGNALING PATHWAY

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Ovarian cancer (OC) recurrence after tumor eradication by chemotherapy invariably heralds poor outcome. Recent data point to persistence of quiescent cancer stem cells (CSCs) not eliminated by chemotherapy and able to regenerate tumors as the main contributor to tumor relapse. Downregulation of tumor suppressor gene (TSG) DAB2IP, a member of the Ras GTPase-activating protein family, significantly correlated with poor patient survival in high-grade serous (HGS) OC. Furthermore, loss of DAB2IP in prostate and colon cancer enriched CSC population, suggesting a key role for DAB2IP in modulating cancer stemness. In the current study, we tested the hypothesis that targeting DAB2IP would inhibit CSCs in OC and prevent disease recurrence. Subpopulations of CSC and non-CSC were isolated from Kuramochi, OVCAR3 and COV362 HGSOC cell lines by fluorescence-activated cell sorting (FACS) based on aldehyde dehydrogenase (ALDH) activity, a consistent CSC marker. It was previously demonstrated by us and others that ALDH(+) cells share characteristics of normal stem cells such as the ability to form anchorage-independent multicellular aggregates (spheroids), undergo membrane efflux, express stem cell restricted transcription factors, and generate tumors in vivo. DAB2IP was examined in the FACS-sorted cells using qPCR and western blot. Expression of DAB2IP in ALDH(+) cells was lower ($P<0.05$) compared to non-CSC ALDH(-) cells. Chromatin immunoprecipitation (ChIP) analysis revealed greater ($P<0.05$) enrichment of H3K27me3 at DAB2IP promoter loci in CSC than non-CSC, suggesting that DAB2IP downregulation in CSC is caused by EZH2 methylation. Enforced overexpression of DAB2IP decreased ($P<0.05$) the number of ALDH(+) cells, inhibited ($P<0.05$) the ability of these cells to form spheroids (14-day incubation under stem cell conditions) and decreased ($P<0.05$) colony formation. Furthermore, elevated DAB2IP expression decreased ($P<0.05$) cisplatin IC50 of both OCSCs and HGSOC cells and inhibited ($P<0.05$) cell migration capacity (Bowden chamber transwell assay), suggesting DAB2IP plays a role in regulating OCSC function. Mechanistically, decreased expression of stemness-related genes in DAB2IP-overexpressing OCSCs was also observed, indicating potential key effectors downstream of DAB2IP. Moreover, dual luciferase reporter assay showed that ALDH1A1 transcription level was significantly repressed ($P<0.05$) in DAB2IP-overexpressing cells. OVCAR3 cells and DAB2IP-overexpressing OVCAR3 cells were further analyzed by RNA-sequencing and bioinformatics. This transcriptome analysis revealed that DAB2IP overexpression resulted in significantly (FDR < 0.05, fold change > 2) altered expression of 449 genes, including markers strongly associated with CSC phenotypes, including down-regulation of ALDH1A1, LGR5, PROM1, TWIST1 and ATP-binding cassette transporters. Differentially expressed genes were subjected to Ingenuity Pathway Analysis (IPA) for upstream regulators. IPA identified Wnt-signaling pathways as top upstream regulators of these differentially expressed genes, suggesting that Wnt-signaling is a dominant pathway mediating the anti-OCSC effects of DAB2IP. In addition, treating cells with Wnt inhibitor decreased ($P<0.05$) the CSC population, colony formation ability and ALDH1 expression. Collectively, our data reveals that DAB2IP plays a critical role in modulating CSC properties via Wnt-mediated signaling pathway, suggesting novel combination treatment strategy targeting OCSCs and thus impacting tumor relapse and chemoresistance in OC.

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SESSION 3: TUMOR MICROENVIRONMENT & IMMUNOLOGY OF OVARIAN CANCER

INVITED SPEAKERS

131 DECONSTRUCTING AND RECONSTRUCTING THE MICROENVIRONMENT OF HIGH-GRADE SEROUS OVARIAN CANCER
Frances Balkwill, PhD • *Barts Cancer Institute (United Kingdom)*

133 MULTI-LEVEL PROTEOMICS IDENTIFIES CANCER TESTES ANTIGENS AS MEDIATOR OF CHEMO SENSITIVITY AND IMMUNOTHERAPY TARGET IN OVARIAN CANCER
Ernst Lengyel, MD, PhD • *University of Chicago*

ABSTRACT PRESENTATIONS

135 MOLECULAR AND FUNCTIONAL HETEROGENEITY OF CANCER ASSOCIATED FIBROBLASTS IN HIGH-GRADE SEROUS OVARIAN CANCER
Laurie Ailles, PhD • *University Health Network (Canada)*

137 PRO-INVASIVE TUMOUR-STROMA INTERACTIONS: ROLE OF THE SECRETED OXIDOREDUCTASE CLIC3
Sara Zanivan, PhD • *CRUK Beatson Institute (United Kingdom)*

139 COLLAGEN ALTERATIONS IN HUMAN OVARIAN CANCER PROBED BY SECOND HARMONIC GENERATION (SHG) IMAGING MICROSCOPY
Paul J. Campagnola, PhD • *University of Wisconsin-Madison*

141 STROMAL CELL EXPRESSION OF THE RECEPTOR TYROSINE KINASE DDR2 PROMOTES OVARIAN CANCER METASTASIS
Katherine Fuh, MD, PhD • *Washington University*

143 A NOVEL TARGET FOR OVERCOMING ADAPTIVE RESISTANCE TO ANTI-ANGIOGENIC THERAPY IN OVARIAN CANCER
Yunfei Wen, PhD • *The University of Texas, MD Anderson Cancer Center*

145 DNA DAMAGE RESPONSES AND IMMUNE PROFILING THROUGH HIGHLY MULTIPLEXED TISSUE IMMUNOFLUORESCENCE (T-CYCIF) IN HIGH-GRADE SEROUS OVARIAN CANCER
Anniina Färkkilä, PhD • *Dana Farber Cancer Institute*

147 EARLY LOSS OF MONOUBIQUITYLATION OF HISTONE H2B ALTERS KEY IMMUNE SIGNALING PATHWAYS PROMOTING THE PROGRESSION OF HIGH-GRADE SEROUS OVARIAN CANCER
Jagmohan Hooda, PhD • *University of Pennsylvania*

149 ALTERNATIVELY ACTIVATED MACROPHAGE SECRETOME MODULATES METASTATIC POTENTIAL OF OVARIAN CANCER CELLS
Kaitlin Fogg, PhD • *University of Wisconsin - Madison*

SESSION 3: TUMOR MICROENVIRONMENT AND IMMUNOLOGY OF OVARIAN CANCER (POSTER PRESENTATIONS)

150 POSTER TMIM–063 | SESSION I: THURSDAY
Targeting Neuropilin-1+ T regulatory cells in patients with high grade serous ovarian cancer decreases Treg-specific suppression of CD8+ T cells
Tullia Bruno, PhD
University of Pittsburgh

151 POSTER TMIM–064 | SESSION II: FRIDAY
AMPKa-like proteins in ovarian cancer tumorigenesis and necroptosis
Ilana Chefetz, PhD
University of Minnesota

152 POSTER TMIM–065 | SESSION I: THURSDAY
Micro-RNA Let-7 Regulation and Function in Ovarian Cancer and Early Embryonic Development
Evgeny A Chirshev, Student
Loma Linda University

153 POSTER TMIM–066 | SESSION II: FRIDAY
EZH2 regulates the epigenetic reprogramming of ovarian cancer promoting carcinoma-associated mesenchymal stem cells
Lan Coffman, MD, PhD
University of Pittsburgh Medical Center

154 POSTER TMIM–067 | SESSION I: THURSDAY
Expression of lncRNAs in ovarian cancer-associated fibroblasts is associated with patient survival
Emily Colvin, PhD
University of Sydney (Australia)

155 POSTER TMIM–068 | SESSION II: FRIDAY
Targeting ER stress responses to unleash therapeutic immunity against ovarian cancer
Juan R Cubillos-Ruiz, PhD
Weill Cornell Medicine, Cornell University

156 POSTER TMIM–069 | SESSION I: THURSDAY
Prospective Clinical Validation of Ex Vivo, Patient-Specific Response Prediction to First-Line Chemotherapy
Tessa DesRochers, PhD
KIYATEC, Inc.

157 POSTER TMIM–070 | SESSION II: FRIDAY
Apelin Promotes Omental Metastasis of Ovarian Cancer Cells
Samrita Dogra, PhD
The Oklahoma University Health Sciences Center

158 POSTER TMIM–071 | SESSION I: THURSDAY
Sox2 drives ST6Gal-I expression and activity to promote a CSC phenotype in ovarian cancer
Kaitlyn Dorsett, BS
University of Alabama at Birmingham

159 POSTER TMIM–072 | SESSION II: FRIDAY
Combinational targeting of HDAC6 and PD-1/PD-L1 immune checkpoint in ARID1A-mutated ovarian cancers
Nail Fatkhutdinov, MS
Wistar institute

160 POSTER TMIM–073 | SESSION I: THURSDAY
The combination of interferons alpha and gamma and monocytes induces ovarian cancer cell death and provide a rationale for a novel, ongoing, immunotherapy Phase 1 clinical trial
Daniel Green, PhD
National Cancer Institute, NIH

161 POSTER TMIM–074 | SESSION II: FRIDAY
IGF2 in human follicular fluid promotes lymphomagenesis in mammary fat pad of Trp53-null mice, depending on estrogen in microenvironment
Hsuan-Shun Huang, PhD, MSc
Research Department/ Hualien TZU CHI Hospital (Taiwan)

162 POSTER TMIM–075 | SESSION I: THURSDAY
Mutant p53 promotes the initiation of high-grade serous ovarian carcinoma from fallopian tube tumor precursors through enhancing tyrosine receptor kinase B signaling
Min Kang, MD
Yale School of Medicine

163 POSTER TMIM–076 | SESSION II: FRIDAY
Omental Macrophages Regulate Ovarian Cancer Metastatic Colonization through the CCL6-CCR1 signaling axis
Venkatesh Krishnan, PhD
Stanford University

164 POSTER TMIM–077 | SESSION I: THURSDAY
Myxoma virus enhances treatment benefit of chemotherapy and dendritic cell immunotherapy in ovarian cancer preclinical model
Jia Liu, PhD
University of Arkansas for Medical Sciences

165 POSTER TMIM–078 | SESSION I: THURSDAY
Using A Dynamic, Low-shear Environment to Mimic Physiological Fluid Shear Stress in Ovarian Cancer in vitro Modeling
Timothy Masiello, MS
SUNY Polytechnic Institute

166 POSTER TMIM–079 | SESSION II: FRIDAY
Inhibition of tumor microenvironment cytokine signaling sensitizes ovarian cancer cells to antiestrogen therapy
Karen McLean, MD, PhD
University of Michigan

167 POSTER TMIM–080 | SESSION I: THURSDAY
Mechanotransduction in ovarian cancers
Geeta Mehta, PhD
University of Michigan

168 POSTER TMIM–081 | SESSION II: FRIDAY
The hypoxic tumor-mesothelial niche promotes ovarian cancer metastasis through collagen remodeling
Suchitra Natarajan, PhD
Stanford University

169 POSTER TMIM–082 | SESSION I: THURSDAY
OvaCure - Bringing personalized treatment to women with ovarian cancer
Adoracion Pegalajar–Jurado, PhD
OvaCure (Denmark)

170 POSTER TMIM–083 | SESSION II: FRIDAY
A Two-Step Targeting Strategy Utilizing Glycoengineered Mesenchymal Stem Cells for Ovarian Cancer
Swayam Prabha, PhD
University of Minnesota

171 POSTER TMIM–084 | SESSION I: THURSDAY
PKCα induces Twist1 phosphorylation at Serine 144 and promotes EMT in ovarian cancer cells
Roslyn Tedja, PhD
Yale University

172 POSTER TMIM–085 | SESSION II: FRIDAY
Bromodomain inhibition in ovarian cancer and the tumor microenvironment
Fiona Yull, DPhil
Vanderbilt University

NOTES

DECONSTRUCTING AND RECONSTRUCTING THE MICROENVIRONMENT OF HIGH-GRADE SEROUS OVARIAN CANCER

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NOTES

MULTI-LEVEL PROTEOMICS IDENTIFIES CANCER TESTES ANTIGENS AS MEDIATOR OF CHEMO SENSITIVITY AND IMMUNOTHERAPY TARGET IN OVARIAN CANCER

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No abstract provided.

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MOLECULAR AND FUNCTIONAL HETEROGENEITY OF CANCER ASSOCIATED FIBROBLASTS IN HIGH-GRADE SEROUS OVARIAN CANCER

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High grade serous ovarian cancer (HGSC) is typically diagnosed at an advanced stage and the vast majority of patients relapse and die within 5 years of diagnosis. Significant clinical problems in HGSC include wide-spread abdominal dissemination of disease and chemotherapy resistance. Cancer-associated fibroblasts (CAFs) have been shown to play a role in promotion of cancer cell proliferation and invasion, and mediation of chemotherapy resistance. To interrogate the molecular properties of CAFs in HGSC we used fluorescence activated cell sorting to isolate CAFs directly from primary tumor samples and performed gene expression profiling. We found that patients stratify into two classes based on their CAF gene signatures: One with high expression of Fibroblast Activation Protein (FAP-High; FH) and one with low expression of FAP (FAP-Low; FL). FH CAFs express classical CAF genes whereas FL CAFs possesses a preadipocyte-like molecular signature. The FL phenotype has remained largely unnoticed as it is generally out-competed in vitro by FH cells when grown under classical CAF culture conditions. Patients from The Cancer Genome Atlas (TCGA), as well as from our own institute, can be stratified into FH and FL subtypes; in both cohorts patients with FH CAFs have a significantly shorter disease-free and overall survival. In vitro and in vivo functional assays performed with isolated CAFs of both types indicate that FH CAFs aggressively promote proliferation, invasion and therapy resistance of cancer cells, whereas FL CAFs do not. Finally, we identified TCF21, a transcriptional repressor, as a FL-specific transcription factor. Analysis of published TCF21 ChIP-Seq data indicates that TCF21 targets a large number of genes specific to FH CAFs. Overexpression of TCF21 in FH CAFs partially reversed their ability to promote cancer cell invasion and tumor growth. Our discovery of CAF heterogeneity in HGSC highlights the need to personalize patient treatment with respect to both cancer and stromal phenotypes. FH patients may benefit from inhibition of cancer-stroma interactions or from epigenetic modulators that reprogram cancer-promoting FH CAFs into the non-supportive FL state.

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PRO-INVASIVE TUMOUR-STROMA INTERACTIONS: ROLE OF THE SECRETED OXIDOREDUCTASE CLIC3

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Cancer cells are embedded within a microenvironment populated with cancer associated fibroblasts (CAFs) with whom they establish two-way communications through paracrine factors and physical interactions. Through these interactions, CAFs play pivotal roles in cancer and have emerged as promising therapeutic target.

To unravel key contributors of CAF-tumour/stroma cell interactions, we have developed mass spectrometry (MS)-based approaches to map proteins secreted in condition medium and extracellular matrix (ECM). Through an extensive MS-proteomic comparative analysis of CAFs with their normal fibroblasts (NFs) counterpart, we have identified the chloride intracellular channel protein 3 (CLIC3), a protein previously considered an intracellular chloride channel (regulator), as one of the most upregulated proteins in CAFs and deposited in the ECM.

Secreted CLIC3 promotes invasive behaviour of endothelial cells to drive blood vessels growth and increases invasiveness of cancer cells, both in vivo and in 3D cell culture models, via activation of the tissue transglutaminase-2 (TGM2). We found that CLIC3 is a glutathione-dependent oxidoreductase that reduces TGM2 and regulates TGM2 binding to its cofactors. Finally, CLIC3 is also secreted by cancer cells, is abundant in the stromal and tumour compartments of aggressive ovarian cancers and its levels in primary tumours and omental metastases correlate with poor clinical outcome.

Our work has unraveled an unprecedented mechanism of cell invasion to be explored for targeting in ovarian cancer.

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NOTES

COLLAGEN ALTERATIONS IN HUMAN OVARIAN CANCER PROBED BY SECOND HARMONIC GENERATION (SHG) IMAGING MICROSCOPY

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PURPOSE: Significant remodeling of the extracellular matrix (ECM) occurs in human ovarian cancer and has been observed in both high grade and low grade tumors as well as ovaries from high risk patients with BRCA mutations. Uniquely quantifying alterations in the tumor microenvironment (TME) could be used as a new diagnostic tool, e.g. to complement histology; provide insight into disease etiology; and assess treatment efficacy through minimally invasive in vivo imaging. Current clinical modalities (CT, MRI, PET) lack the resolution for this purpose. Our efforts have focused on examining collagen alterations across a spectrum of human ovarian tumors, where the changes can serve as quantitative biomarkers. Alterations can be reflected in increased collagen concentration, changes in alignment of collagen molecules within fibrils and/or fibers and/or up-regulation of different collagen isoforms, e.g. Col III.

METHODS AND RESULTS: We used the collagen specific/sensitive Second Harmonic Generation (SHG) imaging microscopy to probe collagen architecture over size scales that range from the macromolecular structural properties to fibril/fiber morphology. First, we used SHG polarization analyses to probe collagen macromolecular/supramolecular properties to discriminate ex vivo human tissues (normal stroma, benign tumors, and high grade serous tumors) by determination of: i) collagen alpha helical pitch angle, ii) alignment of collagen molecules within fibrils, and iii) collagen helical chirality via SHG circular dichroism (SHG-CD). The largest differences were between normal stroma and benign tumors, consistent with gene expression data showing Col III is up-regulated in the latter. The different tissues also displayed differing collagen alignment within fibrils and SHG-CD responses, consistent with either Col III incorporation or randomization of Col I alignment within benign and high-grade tumors fibrils. These results collectively indicate the fibril assemblies are distinct in all tissues and likely result from synthesis of new collagen rather than remodeling of existing collagen. Importantly, these techniques do not require exogenous labels, and additionally, provide sub-resolution structural information previously obtained through ultrastructural analysis that cannot be performed on intact tissues.

We next implemented a novel form of 3D texture analysis and machine learning to delineate the fibrillar morphology observed in SHG images of normal stroma, high risk stroma, benign tumors, and a spectrum of malignant tumors (high grade serous, low grade serous, and endometrioid). We extracted textural features in the 3D image sets to build statistical models of each class and we achieved clinically significant 83-91% classification accuracies for the six classes. Importantly, the 3D analysis significantly outperformed our prior 2D methods. This classification based on collagen morphology will complement conventional classification based on genetic profiles and can serve as an additional biomarker.

CONCLUSIONS: Taken together, the combination of the macro/supramolecular probes and the fiber morphology classification will greatly increase our understanding of the TME evolution in human ovarian cancer. These methods and their findings have clinical translational significance in terms of understanding disease etiology, and also by enhancing prognostic and diagnostic capabilities.

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NOTES

STROMAL CELL EXPRESSION OF THE RECEPTOR TYROSINE KINASE DDR2 PROMOTES OVARIAN CANCER METASTASIS

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OBJECTIVES: Understand the role of stromal discoid domain receptor 2 (DDR2) expression in ovarian cancer metastasis

METHODS: 111 high-grade serous ovarian cancer specimens were examined for stromal DDR2 protein expression by immunohistochemistry (IHC) and correlated with survival. The stromal cell contribution of DDR2 for the steps of metastasis were evaluated through mesothelial cell clearance, Matrigel invasion assays, and germline knock-out mice. DDR2 low versus DDR2 high primary stromal cells were cultured from normal omenutum: human peritoneal mesothelial cells (HPMC) and normal omental fibroblasts (NOF). DDR2 knock-out (KO) and wild-type (WT) mice were used to evaluate metastasis. DDR2 positive (ID8Trp53-/-BRCA2-/- and ES2) tumor cells were used. Mesothelial cell clearance area was evaluated by measuring the size of the clearance area, invasion was evaluated by the number of tumor cells per high power field (hpf), and metastatic spread evaluated by number of tumors nodules and tumor weight. Collagen contribution was evaluated with Masson's trichrome stain and second harmonic generation (SHG). In addition, the media of genetically inhibited DDR2 or therapeutic inhibition of DDR2 in the NOFs (siControl vs siDDR2) was removed and then added to tumor cells to evaluate for invasion. Therapeutic inhibition was performed with a small molecule developed in a collaborating lab, WRG-R28.

RESULTS: Patients with high stromal cell DDR2 expression had a median overall survival (OS) of 171 months vs. low stromal cell DDR2 expression (OS) of 28 months ($p<0.0001$). Mesothelial cell clearance was performed with HPMCsiControl vs siDDR2 and found to have decreased tumor cell clearance with HPMCsiDDR2 when compared to HPMCsiControl. Additionally, tumor cell invasion was decreased by 2-fold in the NOFsiDDR2 compared to NOFsiControl in two discrete NOFs. Furthermore, media from NOFsiControl vs NOFsiDDR2 was removed and co-cultured with DDR2+ tumor cells and found to have 50% less invasion for tumor cells co-cultured with NOFsiDDR2 compared to NOFsiControl. DDR2 KO mice had significantly less tumor weight (0.025g vs 0.05, $p<0.05$) than DDR2 WT mice. Collagen content was found to be less intense at 45% compared to 80%, $p<0.0001$ in the DDR2KO vs DDR2WT. SHG analysis showed DDR2 WT tumors to be more perpendicular than DDR2 KO tumors were more parallel to the tumor-stromal border. Therapeutic inhibition with WRG-R28 treatment of the NOFs resulted in 2-fold decrease in invasive tumor cells (150 vs 75 cells/hpf, $p<0.01$) as well as inhibition of mesothelial cell clearance by the tumor cells treated with WRG-R28 compared to DMSO.

CONCLUSIONS: The stromal contribution of DDR2 promotes tumor cell clearance of mesothelial cells and metastatic spread. This suggests that stromal expression of DDR2 may be a potential target to guide future therapy particularly in the maintenance setting.

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A NOVEL TARGET FOR OVERCOMING ADAPTIVE RESISTANCE TO ANTI-ANGIOGENIC THERAPY IN OVARIAN CANCER

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Anti-angiogenic therapy such as anti-VEGF antibody (AVA) has been being increasingly applied in patients with ovarian cancer, while vast majority developed adaptive resistance, highlighting the need for new therapies. We have screened a cohort of patients with high-grade serous cancer (HGSC) using cDNA-genomic array and immunohistochemistry. Our results indicated that p130cas [Crk-associated substrate], a central node in FAK/Src-mediated angiogenesis, was significantly upregulated in tumor-associated vascular endothelium, and the clinical analysis showed that HGSC patients with increased vascular p130cas have significantly shorter disease-progression survival than those with lower vascular p130cas levels.

Our cell-based studies showed that AVA treatment in endothelial cells led to internalization of a 100-kD fragment of VEGFR2, which was released by caspase-10 cleavage from membrane-tethered VGFR2. This 100-kD form of VEGFR2 bound with TNKS1BP1 (a tankyrase-1-binding protein involved in p53-mediated cell cycle arrest) and internalized into LC3-tagged autophagosomes or translocated into nucleus to initiate cell death. Furthermore, the gene ablation of p130cas with CRISPR/CAS9 in endothelial cells that were originally resistant to AVA therapy re-sensitized them to AVA treatment. Our *in vivo* studies on targeting vascular p130cas with host-specific siRNA showed a robust inhibition of tumor growth and progression in orthotopic HGSC tumors through initiating autophagy-associated cell death in tumor-associated endothelial cells.

To functionally characterize role of vascular p130cas in angiogenesis, we established the p130cas$^{flox/flox}$-Tie2^{Cre} genomic-engineered mice (GEM) model. Using the ID8 syngeneic model, we found that depletion of vascular p130cas diminished resistance to AVA therapy and compromised angiogenesis by inducing elevated VEGFR2/TNKS1BP1 in autophagosomes and nucleus of tumor-associated endothelial cells, which were followed by cell death. To further explore the therapeutic potential of blocking tumor-associated vascular p130cas as a novel anti-angiogenic strategy, we have constituted a cell-permeable, peptide-nanoparticle complex using a p130cas antagonist encapsulated with biodegradable, long-circulating, core-crosslinked polymeric micelles (CCPM). This CCPM-p130cas antagonist contains a mutated Src-binding domain and is linked with Arg-Gly-Asp (RGD) peptide, which specifically targets angiogenic endothelial cells. We also performed the cell-based studies to show that this CCPM-p130cas antagonist is able to bind to the FAK and Src complex with high affinity and effectively block FAK/Src mediated angiogenic property in endothelial cells. Ongoing studies are focused on investigating the therapeutic efficacy and mechanism of actions for RGD-CCPM-p130cas antagonist as a novel anti-angiogenic therapy to overcome adaptive resistance occurred in patients with HGSC.

In summary, our studies provided new knowledge regarding the pivotal role of vascular p130cas in tumor-associated endothelial vasculature, and the critical pre-clinical evidences for applying the RGD-CCPM-p130cas antagonist as a novel therapeutic for treatment of ovarian cancer.

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DNA DAMAGE RESPONSES AND IMMUNE PROFILING THROUGH HIGHLY MULTIPLEXED TISSUE IMMUNOFLUORESCENCE (T-CYCIF) IN HIGH-GRADE SEROUS OVARIAN CANCER

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INTRODUCTION: Immune checkpoint blockade (ICB) has emerged as a new therapeutic approach for multiple cancers, however, the responses to single-agent ICBs have been modest in high-grade serous ovarian cancer (HGSOC). Preclinical and early clinical data indicate promising efficacy of combination with DNA damaging agents and immunotherapy, however lack of functional- and tissue geographical knowledge on the interplay between DNA repair and immune activation has hampered the future development of these strategies. The majority of HGSOC are deficient in homologous recombination (HR) DNA repair, and this deficiency is associated with increased immune recognition and potentially increased response to ICBs. Compelling evidence has shown that DNA damaging agents increase the expression of immune-regulatory genes, such as interferons, which can potentially overcome resistance to ICB. There is a critical need for a deeper understanding of the dynamics between DNA damage in cancer cells and anti-tumor immune responses in HGSOC in order to find rational combinations and predictive biomarkers for DNA damaging agents and immunotherapy.

RESULTS: We are employing a novel, high-multiplex tissue cyclic immunofluorescence (t-CycIF) platform allowing for the simultaneous detection of up to 60 different antigens at single cell resolution. To reveal the effects of between intrinsic and treatment-induced DNA damage in HGSOC, we are profiling the microenvironments in HGSOCs with inherent DNA repair deficiencies, and after DNA damaging therapy. We collected clinically annotated cohorts of 37 BRCA1/2 mutated and 17 HR wild-type patients (Strickland et al, 2016), as well as six paired pre- and post-treatment and 18 post-treatment tumor samples from patients undergoing neoadjuvant chemotherapy (NACT). Using image analysis we generated highly multiplexed single cell data for over 10^6 cells. Through supervised clustering, we evidenced distinct cell compositions in the tumor microenvironment of BRCA1/2 mutated and HR-wild type HGSOCs. Consistent with the role of immune-suppression in HGSOC progression, we found that high infiltration of CD4/FOXP3+ regulatory T-cells associated with more actively proliferating cancer cells. Interestingly, tumors with high expression of PD1/PD-L1 were found to have high infiltration of CD1c+ dendritic cells potentially indicating active suppression of antigen presenting pathways in these tumors. Further, tumors with high levels of DNA damage show active interferon signaling, which associated with significantly higher CD8+ cytotoxic T-cell infiltration. In addition, our preliminary evidence suggests heterogenous DNA damage response- and immune profiles in samples collected after NACT.

CONCLUSIONS: BRCA1/2 mutated tumors have a distinct microenvironment compared to HR-wt HGSOC. In support of earlier findings, FOXP3+ T-cells contribute to immune suppression in HGSOC. The high infiltration of dendritic cells and PD1/PD-L1 expression indicates a subgroup of HGSOC that are likely sensitive to ICBs. Further, increased DNA damage and interferon pathway activation delineated a more immunogenic subset of HGSOC. We conclude that t-CycIF could accelerate the development of rational strategies for combining DNA damaging agents with immunotherapy to ultimately improve the treatment and outcomes of patients with ovarian cancer.

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EARLY LOSS OF MONOUBIQUITYLATION OF HISTONE H2B ALTERS KEY IMMUNE SIGNALING PATHWAYS PROMOTING THE PROGRESSION OF HIGH-GRADE SEROUS OVARIAN CANCER

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High-grade serous ovarian carcinoma (HGSOC) is the most common form of ovarian cancer, accounting for over 70% of all cases. HGSOC is frequently complicated by the inevitable development of therapeutic resistance, and thus remains an incurable disease. Recent insights supporting the fallopian tube epithelium (FTE) as the point of origin for HGSOC and the serous tubal intraepithelial carcinoma (STIC) as the precursor lesion for a majority of HGSOCs provide the necessary context to study the mechanisms that drive the development and progression of HGSOC. Understanding these key molecular processes is essential to inform the development of next generation therapies. In this study we investigated the role of the ubiquitin ligase RNF20 and histone H2B monoubiquitylation (H2Bub1) in serous tumorigenesis. H2Bub1, catalyzed largely by RNF20, is an epigenetic mark with tumor suppressor properties. The loss of RNF20/H2Bub1 has been linked with cancer progression. We used immunohistochemistry to characterize the expression of H2Bub1 in HGSOC and FTE precursors. We found that H2Bub1 is lost or downregulated in a large proportion of STICs and HGSOC tumors, implicating RNF20/H2Bub1 loss as an early event in serous tumorigenesis. Consistent with our findings, analysis of TCGA data shows that the majority of HGSOCs exhibit heterozygous loss of *RNF20*. Functionally, we demonstrate that shRNA-mediated knockdown of RNF20, with concomitant loss of H2Bub1, is sufficient to increase cell migration and clonogenic growth, both in 2D and 3D, of immortalized FTE cells. To understand the mechanisms underlying these effects, we performed ATAC-seq and RNA-seq in *RNF20* knockdown cells. Interestingly, major changes were observed in a number of immune signaling pathways, providing early mechanistic insights for the observed oncogenic phenotypes. Using ELISA we confirmed the upregulation of IL6 and other cytokines in RNF20 knockdown cells. Additionally, we confirmed that the oncogenic phenotype, enhanced migration, is in part driven by the increased levels of IL6 in RNF20 and H2Bub1 knockdown cells which can be neutralized by using an anti-IL6 antibody. In summary, our study identifies that loss of H2Bub1 is an early epigenetic event in HGSOC that rewires certain immune signaling pathways and may represent unique therapeutic opportunities.

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ALTERNATIVELY ACTIVATED MACROPHAGE SECRETOME MODULATES METASTATIC POTENTIAL OF OVARIAN CANCER CELLS

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High-grade serous ovarian cancer (HGSOC) metastasizes when tumor spheroids detach from the primary tumor and re-attach throughout the peritoneal cavity. Once spheroids have attached in a new site, the top layer of mesothelial cells is lost and the cancer cells expand across the underlying extracellular matrix (ECM). The factors that influence this process are unclear, but may provide therapeutic targets to slow disease spread. As HGSOC progresses an increase in alternatively activated macrophages (AAMs) in the surrounding ascites fluid has been observed and AAMs enhance tumor invasion and growth in a wide range of cancers. Thus we hypothesized that soluble factors from the AAMs in the peritoneal microenvironment promote the disaggregation of HGSOC spheroids across the underlying ECM.

In order to model interactions between primary human AAMs and HGSOC spheroids (OVCAR3, OVCA433, OV90), an in vitro micro-culture device was used that allows for the examination of paracrine signaling in a controlled environment. After two days of co-culture on an 8 mg/mL collagen I hydrogel the area covered by the HGSOC cells was measured and normalized to the initial area of the spheroid. We determined that co-culture with AAMs significantly increased HGSOC spheroid spreading across the collagen matrix for all the HGSOC cell lines examined. To identify the AAM-derived soluble factors responsible for this increased spreading, media was collected from the device and screened for 35 cytokines using a Bioplex assay. The correlation between spheroid spreading and AAM-derived soluble factors was examined using PLSR; a one component PLSR model captured the co-variation between soluble factors and spheroid spreading (R2Y = 0.9) and was able to predict spheroid spreading given the cytokine profile (Q2Y = 0.66). Analysis of the PLSR model identified five ligands (Flt-3L, HB-EGF, IL-6, IL-8, and leptin) that strongly correlated with increased spheroid spreading. These findings were experimentally validated by adding recombinant versions of these proteins in the absence of AAMs as well as inhibiting the receptors for these factors in the presence of AAMs. From these experiments we discovered that each of the HGSOC cell lines was responding to a different AAM-secreted factor; Flt-3L induced spreading in the OVCAR3 cell line, leptin induced spreading in the OVCA433 cell line, and HB-EGF induced spreading in the OV90 cell line. The downstream pathway of these ligands was then interrogated by adding growth factors that induced spreading in combination with cucurbitacin-I, a selective JAK2/STAT3 inhibitor, ab142180, a selective MMP9 inhibitor, or verteporfin, a YAP inhibitor. The effect of these stimulants was inhibited by both cucurbitacin-I and ab142180 yet not by verteporfin, indicating that the AAM-derived factors utilize a common signaling pathway to mediate their effect on spheroid spreading: JAK2/STAT3 activation followed by MMP-9 mediated spreading.

These data suggest that inhibiting singular soluble factors will not inhibit AAM-induced effects across a broad group of patients and that downstream pathways should instead be examined as potential therapeutic targets to slow metastasis in HGSOC.

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TARGETING NEUROPILIN-1+ T REGULATORY CELLS IN PATIENTS WITH HIGH GRADE SEROUS OVARIAN CANCER DECREASES TREG-SPECIFIC SUPPRESSION OF CD8+ T CELLS

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Regulatory T cells (Treg) are a subpopulation of CD4+ T cells that suppress autoimmune responses, but also prevent clearance of tumors and chronic viral infections. In high grades serous ovarian cancer, a higher frequency of tumor infiltrating (TIL) Treg are associated with poor prognosis, but mechanisms governing the suppressive activity of TIL Treg in this context are limited. Our group has recently described a signaling axis through neuropilin-1 (NRP1) on TIL Treg in murine models of cancer that promotes the survival and suppressive function of Treg. When NRP1 is specifically knocked out on murine TIL Treg, there is reduced tumor growth and increased survival similar to the complete knockout of TIL Tregs, however, without death from overt autoimmunity. Thus, NRP1 is a viable target to specifically reduce TIL Treg suppressive function in the tumor microenvironment (TME), ultimately leading to increased anti-tumor immunity without adverse events. Given the importance of NRP1+ TIL Treg in murine cancer models, we sought to understand the role of NRP1 on TIL Treg from patients with high grade serious ovarian cancer.

Surface and total NRP1 expression was assessed by flow cytometry on CD4+CD25+CD127loFOXP3+ Treg from healthy donor PBL and ovarian cancer TIL or ascites fluid. Total NRP1 was expressed on a median of 1.8% (interquartile range [IQR]: 0.69% to 4.8%) of Treg from healthy donors compared with a median of 66% (IQR: 28% to 90%; p<0.001) and 82% (IQR: 38% to 99%; p=0.004) of Treg from ovarian TIL and ascites, respectively. Surface NRP1 was detected on 0.1% (IQR: 0% to 1%) of Treg in PBL from healthy donors, compared with 11.1% (IQR: 1.1% to 60%; p=0.01) on ovarian TIL and 82% (IQR: 14% to 82%; p=0.0015) on ascites. Further, in comparing expression of NRP1 on Treg from benign ovarian disease and malignant ovarian tumors, there was a trend toward increased NRP1 expression on Tregs from malignant disease (median 9.3% versus 54.5%, respectively; p=0.044). Finally, NRP1+ TIL Treg isolated from primary tumors or ascites fluid were capable of suppressing CD8+ T cell proliferation in a dose-dependent manner and suppression was abrogated by addition of an anti-NRP1 antibody.

In summary, both surface and total NRP1 are expressed more frequently on Treg from TIL and ascites fluid compared to Treg from healthy donor PBL and NRP1+ Tregs in the TME are more suppressive compared to NRP1- Tregs. NRP1 is a viable target to reduce Treg suppressive function in ovarian cancer tumors, which can be immunologically ""cold"". This could ultimately lead to increased CD8+ T cell infiltration and function in these patient tumors, which could then increase responsiveness to anti-PD1 as it relies upon CD8 T cell infiltration to be effective.

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AMPKA-LIKE PROTEINS IN OVARIAN CANCER TUMORIGENESIS AND NECROPTOSIS

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In our previous research we characterized novel ALDH1A inhibitors (1A1, 1A2, 1A3) 673A that specifically target Ovarian Cancer Stem cells (OvCSC) and trigger cell programmed necrosis (necroptosis). Following ALDH1A inhibition CD133+ (A2780) FACS sorted cells we detected increase in phospho LKB1 while its major downstream target AMPKa did not exhibit changes in phosphorylation level. In addition to AMPKa, which is most known downstream target of LKB1, there are 11 AMPKa-like proteins that can be phosphorylated by LKB1. These proteins have been studied mostly in brain development.

To detect AMPKa-like relevancy to high grade serous ovarian cancer, we next analyzed TCGA data. Surprisingly we identified many alterations in all 12 genes, including LKB1 (STK11).

We only focused on alterations that exhibit gene amplifications or mRNA upregulation. Based on TCGA data, all AMPKa-like genes have many alterations in ovarian tumors suggesting importance of these genes to ovarian cancer tumorigenesis. We next continued to analyze combinations of any two genes to test their co-occurrence or mutual exclusivity. We detected 9 gene combinations, in which amplification or mRNA upregulation in AMPKa-like genes co-occur (p<0.5) in patient tumors. We next validated expression of AMPKa-like genes in ovarian cancer cell lines and confirmed their expression in PEO-4, Ovcar8, and Ovsaho. We next treated Ovsaho, PEO4 and OvCAR8 cells with ALDH1A inhibitor 673A for 8 and 14 hr, and profiled expression of AMPKa-like genes following ALDH1A inhibition. We detected significant changes in AMPKa-like genes, suggesting their role in cell programmed necrosis.

To confirm our bioinformatic findings, we established cell lines that mimic patient tumors with most frequent co-occurrence of AMPKa genes. We have been studying these cell lines in terms of cell proliferation, stemness and role in cell death.

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MICRO-RNA LET-7 REGULATION AND FUNCTION IN OVARIAN CANCER AND EARLY EMBRYONIC DEVELOPMENT

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INTRODUCTION: Epithelial ovarian cancer (EOC) is the fifth deadliest malignancy in women in the United States, with a high death-to-incidence ratio and five-year survival of 30%. Aggressiveness and recurrence of EOC has been attributed to cancer stem cells (CSC) within tumors, which contribute to chemoresistance and relapse. Deregulation of miRNAs has been linked to cancer initiation, progression, and the stem cell state. We focus on let-7 miRNA, which is repressed in many cancers and is associated with decreased survival. Let-7 is essential for cell differentiation, and its repression is required for reprogramming. Decreased let-7 level in cancer is associated with stemness.

OBJECTIVE: Purpose for our research is to understand let-7 expression and function in EOC cell-lines and patients-derived samples in order to understand mechanisms of its abnormal regulation and develop novel treatments.

MATERIALS AND METHODS: Patient-derived samples are used along with EOC cell-lines. Over-expression of Let-7 is achieved by mimic transfection and confirmed via RT-qPCR. Level of miRNA and pluripotency markers mRNA is detected via RT-qPCR, and protein expression confirmed by Western blot. Functional assays to analyze cancer phenotype include wound healing, invasion and spheroid formation assays. Embryoid body (EB) formation is used to mimic murine early embryonic development and guided toward primitive streak via cytokines. Let-7 expression is analyzed via RT-qPCR.

RESULTS: We demonstrate that patient-derived EOC samples have decreased Let-7 levels and increased expression of pluripotency markers, therefore, we hypothesize that up-regulation of let-7 in EOC will decrease aggressiveness and increase chemosensitivity. We have demonstrated that up-regulation of let-7 via mimic transfection reduced cancer stem cell properties demonstrated by reduction of pluripotency markers and spheroid formation. Let-7 also decreased in vitro migration and invasion. Our data show that let-7 suppresses stem cell-like phenotype of EOC, indicating that it may be a treatment option in conjunction with conventional chemotherapies. In order to understand deregulation of let-7 in cancer, understanding its normal regulation and function during development is essential. By using mESCs we demonstrate that let-7 is dynamically expressed, contradicting conventional belief that let-7 levels slowly increase upon differentiation. Previously published data demonstrating that Hmga2, a let-7 target, peaks and is required upon exit from pluripotency supports our results.

CONCLUSION: In conclusion, let-7 is repressed in EOC, and its over-expression demonstrates tumor suppressive functions via decreasing stemness, migration, invasion, and spheroid formation. During development let-7 is dynamically expressed, demonstrating complex regulation.

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EZH2 REGULATES THE EPIGENETIC REPROGRAMMING OF OVARIAN CANCER PROMOTING CARCINOMA-ASSOCIATED MESENCHYMAL STEM CELLS

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Ovarian tumor cells reside within a complex tumor microenvironment (TME) critical to the formation and function of ovarian cancer. Carcinoma-associated mesenchymal stem cells (CA-MSCs) are stromal progenitor cells within the TME which significantly enhance ovarian tumor cell growth and chemotherapy resistance. We demonstrated that CA-MSCs arise from ovarian tumor cell mediated reprograming of normal tissue MSCs. The resulting CA-MSC phenotype has a unique and durable expression profile without acquisition of genetic mutations, suggesting epigenetic mechanisms underpin the induction of a CA-MSC. In support of this, EPIC methylation array and ATACseq data demonstrate CA-MSCs have significant global hypermethylation and overall “closed” chromatin structure compared to normal MSCs. Further, promoter methylation alterations correlate with the gene expression profile which characterize CA-MSCs. We thus sought to investigate the mechanism underlying the cancer-mediated epigenetic reprograming of CA-MSCs.

The histone-lysine N-methyltransferase Enhancer of Zeste homolog 2 (EZH2) is induced early in the conversion of a CA-MSC through tumor secreted factors. EZH2 promotes transcriptional repression through histone H3 lysine 27 trimethylation (H3K27me3) leading to a closed chromatin state. EZH2 also acts as a scaffold for DNA-methyltransferases (DNMTs) thus enhancing DNA methylation. Western blotting confirmed increases in EZH2 and the EZH2 target repressive marks H3K27me3 and H2AK119ub in CA-MSCs versus MSCs. We therefore hypothesize that ovarian cancer mediated induction of EZH2 in normal tissue MSCs is critical to the formation of a CA-MSC through epigenetic reprogramming.

Normal tissue MSCs derived from benign human ovary or omentum and CA-MSCs derived from high grade serous ovarian cancer patient samples were isolated as previously described. To model the tumor-mediated induction of CA-MSCs, direct co-culture of normal tissue MSCs with high grade serous cell lines CAOV3, PEO1 or OVCAR3 were used. MSC/tumor cell co-cultures were treated with the EZH2 inhibitor Tazemetostat and/or the DNMT inhibitor 5-Azacitidine. A mathematical model, referred to as the CA-MSC classifier which uses the expression of 6 genes to accurately distinguish normal MSCs from CA-MSCs, was utilized to assess the impact of EZH2 and DNMT inhibition on the formation of a CA-MSC. In this model, values closest to 1 indicate a high likelihood of being a CA-MSC (a value of >0.8 meets the threshold for a CA-MSC and <0.2 meets the threshold for a normal MSC).

Pharmacologic EZH2 inhibition significantly decreased the induction of a CA-MSC phenotype in tumor cell co-culture without impacting tumor cell or CA-MSC viability. DNMT inhibition alone yielded a modest decrease in CA-MSC induction. However, the combination of EZH2 inhibition and DNMT inhibition blocked the formation of a CA-MSC with a classifier score going from 0.9 (in untreated co-cultures) to 0.1 in dual-inhibition co-cultures.

Collectively, our data suggests that ovarian tumor cell mediated EZH2 induction plays a critical role in the conversion of a MSC into a CA-MSC through epigenetic reprogramming. This reprogramming can be interrupted through targeting EZH2 and DNMT. This presents a novel target to block the formation of the tumor enhancing stromal niche thus offering a potentially powerful new approach to the treatment of ovarian cancer.

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EXPRESSION OF LNCRNAS IN OVARIAN CANCER-ASSOCIATED FIBROBLASTS IS ASSOCIATED WITH PATIENT SURVIVAL

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BACKGROUND: Ovarian cancer is the most lethal gynecological malignancy in women, with high-grade serous ovarian cancer (HGSOC) the most common and aggressive subtype. The tumor microenvironment is acknowledged to play a vital role in the growth and metastasis of many solid tumors, including ovarian cancer, and as such represents an attractive new therapeutic target. In ovarian cancer, patients with a higher proportion of desmoplasia have a poorer survival. Cancer-associated fibroblasts (CAFs) represent the most abundant cell type in the tumor stroma and are responsible for producing the desmoplastic reaction that is a poor prognostic factor in HGSOC. Genetic aberrations in ovarian CAFs are extremely rare, raising the possibility of alternative mechanisms that regulate gene expression in CAFs, such as regulation by long non-coding RNAs (lncRNAs). LncRNAs are transcripts that do not encode for protein, but have been shown to play important roles in several diseases, including cancer. However, very little is known about the role of lncRNAs in the tumor microenvironment.

OBJECTIVES: To identify lncRNAs whose expression levels in CAFs are associated with patient survival and use computational approaches to predict their function.

METHODS: CAFs were laser capture microdissected from 67 advanced stage HGSOCs. RNA was extracted from the microdissected samples and expression analyzed using Affymetrix U133 Plus 2.0 Arrays. Probes identified as lncRNAs were used in this analysis. Samples were normalized and background corrected using the robust multiarray average (RMA) method and expression values were log2 transformed. Expression levels of each lncRNA were clustered into low and high expression groups. Kaplan Meier /log-rank analysis was used to assess the association between expression levels of each lncRNA and the patients' overall survival. Multivariate cox regression analysis was used to determine if differential expression of lncRNAs were independent predictors of survival. A network based 'guilt-by-association' approach was used to predict the function of lncRNAs associated with patient survival.

RESULTS: Increased expression of 9 lncRNAs including *DANCR*, *MALAT1* and *NEAT1* and decreased expression of 1 lncRNA in ovarian CAFs were found to be associated with poorer overall survival by the log-rank test. Expression profiles of 5 lncRNAs as well as response to chemotherapy and debulking status were significant predictors of survival by univariate cox proportional hazards analysis. To adjust for existing collinearity of the 10 lncRNAs, the first principal component of these lncRNAs (capturing 98% of variations), as well as response to chemotherapy and debulking status were incorporated into a multivariate model. The first principal component (HR=0.74, P=0.0001163) and response to chemotherapy (HR=0.22, P=0.000168) were found to be independent predictors of survival. Functional enrichment analysis revealed these lncRNAs are likely to play a role in metabolism, autophagy or immune response.

CONCLUSIONS: We have identified several lncRNAs whose expression levels in CAFs are associated with survival of HGSOC patients, raising the likelihood that they play an important role in the tumor-promoting functions of CAFs. A further understanding of the role of lncRNAs in CAFs may be useful when designing novel therapies that target the tumor microenvironment.

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TARGETING ER STRESS RESPONSES TO UNLEASH THERAPEUTIC IMMUNITY AGAINST OVARIAN CANCER

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Harnessing the intrinsic ability of our immune system to eliminate malignant cells represents the most promising new anti-cancer strategy since the development of chemotherapy. While cancer immunotherapy has been designated a scientific breakthrough, tumor-induced immunosuppression still represents a major impediment to the success of this new approach in the vast majority of cancer patients. We found that ovarian cancer inhibits the development of protective anti-tumor immunity by inducing a state of continued "Endoplasmic Reticulum (ER) Stress" in tumor-associated dendritic cells (tDCs). Sustained activation of the IRE1-XBP1 arm of the ER stress response in DCs was necessary for the aggressive and accelerated progression of primary and metastatic ovarian cancers in various preclinical models of disease. Mechanistically, hyperactive XBP1 disrupted crucial metabolic pathways in tDCs and caused severe immune cell dysfunction at tumor sites. Accordingly, DC-specific XBP1 deletion or selective nanoparticle-mediated XBP1 silencing in tDCs restored their immunostimulatory activity in situ and extended survival by evoking protective T cell-mediated anti-tumor immunity. Our findings uncover a new regulatory role for XBP1-driven signaling in DCs of the tumor microenvironment and suggest that targeting aberrant ER stress responses may represent a new strategy to unleash protective immune responses against ovarian cancer.

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PROSPECTIVE CLINICAL VALIDATION OF EX VIVO, PATIENT-SPECIFIC RESPONSE PREDICTION TO FIRST-LINE CHEMOTHERAPY

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PURPOSE: Ovarian cancer remains the most lethal gynecological malignancy in the United States even though there are a number of treatment options. For the newly diagnosed ovarian cancer patient, treatment is standard with the majority of patients receiving first-line platinum/taxol treatment following surgical debulking or as part of a neo-adjuvant treatment regimen. Standard response assessment to this chemotherapy combination depends on lengthy clinical follow-up of at least 6 months following the conclusion of the chemotherapy. This course is initially effective in 70-80% of patients, but 20-30% will relapse within the 6 months post-chemotherapy or not respond at all. Additionally, most of the initial responders will also relapse. Early prediction of the patients whose cancers do not respond could lead to earlier intervention with more effective therapy.

RESULTS: To perform this prediction, we developed a test utilizing live tumor tissue and 3D cell culture to predict the response of ovarian cancer patients to standard platinum and taxol chemotherapy along with the standard agents used for relapse patients. To validate the assay, we performed a prospective trial involving 86 women with newly diagnosed ovarian cancer from whom live tumor tissue was recovered at surgical resection prior to chemotherapy. Of the 79 patients whose tissues yielded a successful assay result, 27 had sufficient clinical follow-up to determine the accuracy of the response predictions made by the assay. Assay response predictions were made within 7 days following surgery while clinical response was not determined until 6 months or more following the conclusion of first-line chemotherapy. Assay results predicted first-line chemotherapy response in 100% of clinical responders (19 of 19) and first-line chemotherapy non-response in 62.5% of clinical non-responders (5 of 8) as defined by CA-125 and radiographic surveillance. This response prediction was statistically significant by Fisher's Exact test, $p < 0.001$. The sensitivity of the assay was 86% while the specificity was 100%. When the assay results were examined in terms of progression free survival, 19 of 19 patients identified as responders by the assay remained progression free at least 6 months post-chemotherapy with 14 remaining progression free 10 or more months post-chemotherapy. In contrast, of the 8 assay non-responders, 3 were refractory with progression within 3 months of chemotherapy conclusion and 2 progressed less than 6 months post-chemotherapy with a mean progression free survival of 4.5 months.

CONCLUSIONS: Our rapid, ex vivo, 3D cellular assay is capable of accurate patient specific response prediction of first-line combination therapy in newly diagnosed ovarian cancer patients and can serve as a prognostic indicator of progression free survival. This preliminary clinical validation shows the significant potential of this assay as a future tool in personalized cancer care for ovarian cancer patients.

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APELIN PROMOTES OMENTAL METASTASIS OF OVARIAN CANCER CELLS

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PURPOSE: Ovarian cancer (OvCa) is mainly metastasized to the peritoneal cavity and omentum, an organ primarily composed of adipocytes. Adipocytes produce adipokines including apelin involved in initiating angiogenesis and promote the initial homing of tumor cells to omentum through adipokine secretion. Apelin and its receptor APJ mediates glucose/energy metabolism and angiogenesis. The study objective is to assess the functional role of apelin/APJ pathway in omental metastasis of OvCa and associated benefits from antagonizing this pathway as a novel treatment strategy against OvCa metastasis.

METHODS: Mouse adipocyte cell line 3T3-L1 were used to obtain adipocyte-derived conditioned media (adipo-CM). Apelin concentrations in adipo-CM were quantified using Western blot and ELISA. Migration and invasion of human OvCa cell lines (OVCAR-5^{APJ} and OVCAR-8) were determined *in vitro* in the presence of adipo-CM. *In vivo* homing and *ex vivo* adhesion assays were performed to study the role of apelin/APJ pathway in attracting and attaching APJ-overexpressing OvCa cells to the omentum.

RESULTS: Apelin expression was 2.5 fold higher in mature adipocytes compared to that in pre-adipocytes. Mature adipocytes secreted 0.8-1 ng/mL of apelin in the CM. *In vivo*, high APJ expression increased 'homing-in' of OvCa cells by 2.75-fold to the omentum. Adipo-CM increased transwell migration (3-3.5 fold) and invasion (3.5-4 fold) of OvCa cells *in vitro*. Adhesion of APJ high expression OvCa cells to mice omentum *ex vivo* increased by 1.5 fold. These apelin-induced pro-metastatic effects were reversed by apelin-specific antagonist (F13A) in a dose-dependent manner.

CONCLUSION: We found that apelin/ APJ pathway potentially promotes OvCa metastasis to omentum and F13A effectively inhibited apelin-induced effects.

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SOX2 DRIVES ST6GAL-I EXPRESSION AND ACTIVITY TO PROMOTE A CSC PHENOTYPE IN OVARIAN CANCER

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This study elucidates ST6Gal-I, a sialyltransferase, as a functional driver of a cancer stem cell (CSC) phenotype regulated by the stem cell transcription factor Sox2. ST6Gal-I is upregulated in 98% of ovarian cancers (OC) and its high expression correlates with reduced overall and progression free survival in high-grade serous ovarian carcinoma. ST6Gal-I functions to add an α2-6 sialic acid, a large, negatively charged sugar, to N-glycosylated proteins bound for the cell surface. Normal differentiated epithelia have very low expression of ST6Gal-I, however, expression is turned on in stem cell compartments and transformed tissues. Furthermore, our work has shown that ST6Gal-I plays a causal role in conferring hallmark CSC properties including greater tumor-initiating capabilities, and resistance to tumor-associated stressors like chemotherapies, serum deprivation, and hypoxia. However, despite this stark upregulation and functional importance of ST6Gal-I in cancer, very little work has been done to identify the transcriptional drivers of ST6Gal-I expression. We identified that Sox2 and ST6Gal-I are both located on one of the most commonly enriched amplicons in human cancer, amplicon 3q26. We then examined the TCGA databases and found that these two genes are co-amplified in 48/73 cancer cohorts, including ovarian cancer. Furthermore, in those 48 cohorts with co-amplification, there is also co-occurrence per individual patient samples. In addition to being genetically co-amplified, we identified Sox2 response elements in the ST6Gal-I promoter and performed a chromatin immunoprecipitation assay (ChIP) to confirm Sox2 binding. These data implicated Sox2, a key stem-associated transcription factor in CSCs, as a transcriptional activator of ST6Gal-I expression. To confirm this hypothesis, Sox2 was overexpressed or knocked-down in ovarian cancer cells, and it was consistently found that high expression of Sox2 directly induces expression of ST6Gal-I mRNA and protein. Finally, given the relationship between Sox2 and ST6Gal-I in the promotion of a CSC phenotype, we profiled the reprogramming of the cell population into a more stem-like state by quantifying expression of stemness markers Oct4 and Nanog. Overexpression or knockdown of Sox2 resulted in up- or down- regulation of CSC markers Oct4 and Nanog, respectively. Importantly, forced ST6Gal-I knockdown in cells with high Sox2 inhibited the Sox2-induced ST6Gal-I upregulation and subsequently prevented the enhanced expression of Oct4 and Nanog. These data suggest that Sox2 requires ST6Gal-I expression to promote a CSC phenotype. Collectively, our results highlight a novel, glycosylation-dependent mechanism that drives a CSC phenotype.

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COMBINATIONAL TARGETING OF HDAC6 AND PD-1/PD-L1 IMMUNE CHECKPOINT IN ARID1A-MUTATED OVARIAN CANCERS

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Ovarian clear cell carcinoma (OCCC) typically has a low response rate to platinum-based standard of care, and has no effective therapy for advanced stage disease. About 50% OCCCs harbor a mutation in ARID1A, a subunit of a SWI/SNF chromatin remodeling complex. We have previously demonstrated the dependence of ARID1A-mutated OCCCs on HDAC6 activity. In addition, ARID1A inactivation impairs mismatch repair, thus sensitizing ARID1A-mutated tumors to checkpoint blockade. However, anti-PD-L1 treatment only marginally improved the survival of ARID1A-deficient mice. We sought to determine whether combinational approach will improve efficacy of checkpoint blockade therapy in ARID1A-mutated OCCC. We combined HDAC6 inhibitor ACY1215 and PD-1/PD-L1 immune checkpoint blockade utilizing Arid1a-/-/Pik3caH1047R genetic ovarian clear cell model. Combination further prolonged mice survival as compared to single treatments and observed effects correlated with an increase in the activity of anti-tumor cytotoxic T-cells. In addition, we observed increase in dendritic cell infiltration following HDAC6 inhibition, suggesting of an increase in antigen presentation. Our findings indicate that combination of HDAC6 inhibition with PD-L1 checkpoint blockade could be a potential strategy for ARID1A-mutated OCCCs, not only eliminating ARID1A-mutated tumor cells but also restoring anti-tumor immunity.

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THE COMBINATION OF INTERFERONS ALPHA AND GAMMA AND MONOCYTES INDUCES OVARIAN CANCER CELL DEATH AND PROVIDE A RATIONALE FOR A NOVEL, ONGOING, IMMUNOTHERAPY PHASE 1 CLINICAL TRIAL

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Standard of care for the treatment of ovarian cancer is surgical tumor debulking, followed by administration of a platinum based compound in combination with a taxane compound. While there is an initial good response to the therapy, especially in optimally debulked tumors, the disease is characterized by a high rate of relapse. There is no definitive second line treatment for patients. Ovarian cancer is largely retained in the peritoneal cavity, with metastases outside of the peritoneum occurring late in the course of the disease. The restriction of the bulk of the tumor burden to the peritoneal cavity makes intraperitoneal (IP) treatment a reasonable approach for ovarian cancer. This strategy was first employed for ovarian cancer using immunotherapy with IP administration of Interferon Alpha. However, one of the hallmarks of ovarian cancer a highly immunosuppressive environment. This environment includes the metastases themselves and the fluid of the peritoneum which contains a mix of pro and anti-inflammatory cells, cytokines and lipids. Tipping the balance towards a pro-inflammatory environment is necessary for the effective treatment of disease.

Herein, we define the mechanisms by which IFNs and monocytes are potent killers of ovarian cancer cells. While patients with ovarian cancer have normal whole blood counts, the tumoricidal activity of their monocytes has never been measured. We demonstrate that ovarian cancer patient monocytes are more tumoricidal when cultured with IFNs than monocytes from sex and age matched controls. In this work, we expand on our previous observations of synergistic killing of ovarian cancer cell lines by monocytes and IFNs by showing that an important mechanism of cell death is mediated by TRAIL expressed on monocytes, and the target cells die in a Caspase-8 dependent mechanism. We also found that the tumoricidal effect of IFNs and monocytes was independent of IRF9 and STAT2 signaling, and was instead dependent on IRF-1 and STAT1 signaling. Together, these data support a new, innate immune based, approach to immunotherapy of ovarian cancer.

We are currently determining the safety of using autologous monocytes treated ex vivo with IFNs and infused into the peritoneal cavity of patients with advanced ovarian cancer in a phase 1 clinical trial (NCT02948426). While the data presented here and previously published works show that innate mediators of the immune system can kill ovarian cancer cells and decrease disease burden, a durable clinical response is dependent on a strong adaptive immune response. In the clinical trial we will identify whether the highly pro-inflammatory properties of the combination of the innate immune effectors monocytes and IFNs can stimulate an existing, but tumor suppressed, adaptive anti-tumor immune response. Here we present a mechanistic understanding of how IFNs and monocytes can kill ovarian cancer cells and provide mechanistic insights into innate immune based immune therapy for the treatment of ovarian cancer.

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IGF2 IN HUMAN FOLLICULAR FLUID PROMOTES LYMPHOMAGENESIS IN MAMMARY FAT PAD OF TRP53-NULL MICE, DEPENDING ON ESTROGEN IN MICROENVIRONMENT

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Ovulation overdrive is the most important risk factor of ovarian cancer. In previous study we discovered that preovulatory follicular fluid (FF) collected from IVF women, upon weekly injections into the mammary fat pad of Trp53-null mice for 7 weeks, could induce early onset of B cell lymphomas. In subsequent studies (Abstract by Chu TY et al.), we disclosed the IGF-axis signaling conferred by FFs is responsible for the stemness, clonal expansion and transformation of fallopian tube fimbrial epithelial cells where IGF2 in FF bound IGF-1R on secretory cells to activate the downstream PI3K/Akt/mTOR and PI3K/Akt/NANOG signaling pathways for the transformation.

In this study, we identified the same IGF2 is also responsible for the FF-induced lymphomagenesis in Trp53-null mice. When IGF2 was depleted from FF, the tumorigenesis rate reduced from 10/18 (56%) to 1/6 (17%). On the other hand, injections with pure IGF2 (100 mg/ml) grew tumors in 33% (2/6) of mice. The same injection to Trp53 wild type did not grow tumor.

We also found a microenvironment of adipose tissue seemed to be important for the FF-induced lymphomagenesis. The same injections of FF into the subcutis and other sites did not grow tumor.

Additionally, tumor development depends not only on the injection site but also on the female sex hormone and ER function. No tumor growth was observed in castrated female mice and in male mice. Adding fulvastrant to the injections resulted in complete shrinkage of tumor. Meanwhile, extended injection of FF from 7 weeks to 13 weeks promoted tumor progression but did not increase tumor incidence.

The findings suggest a transformation activity of human preovulatory FF largely conferred by IGF2. In the context of germline Trp53 loss, this activity likely promotes expansion of the lymphocytic progenitors that have already been transformed. Meanwhile, we found ER is expressed in the tumor adjacent adipocytes but not in the lymphoma cells. Also, ER antagonist, although largely confined tumor growth, did not decrease the tumor incidence. In this tumorigenesis model conferred by Trp53 loss and FF-IGF2, estrogen seems to play an essential role in tumor progression but not in tumor initiation, and this promotion effect is through the adipocyte microenvironment.

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MUTANT P53 PROMOTES THE INITIATION OF HIGH-GRADE SEROUS OVARIAN CARCINOMA FROM FALLOPIAN TUBE TUMOR PRECURSORS THROUGH ENHANCING TYROSINE RECEPTOR KINASE B SIGNALING

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Lesions in the fallopian tube (FT) fimbriae are hypothesized to be the precursors of high-grade serous ovarian carcinomas (HGSOC). Immunohistalchemical and sequencing analyses demonstrated that FT lesions shared the same TP53 mutation as the surrounding carcinomas. Analyses of mutations and chromosomal alterations in FT lesions, ovarian cancers, and metastases also revealed that the mutation of TP53 is an early event that might drive tumor initiation. Our study focuses on understanding the molecular mechanisms of mutant p53 promoting tumor initiation from the FT.

Our previous data showed that the FT epithelial cells expressed TrkB. The ligand of TrkB, brain-derived neurotrophic factor (BDNF), is secreted by the ovary, omental adipocytes, peritoneal visceral epithelial cells, and immune cells in the omentum and peritoneum. BDNF suppresses anoikis, the apoptosis induced by lack of proper cell to extra-cellular matrix (ECM) attachment. BDNF/TrkB signaling has been associated to tumor progression. We hypothesize that mutant p53 enhances tyrosine receptor kinase B (TrkB) signaling in FT tumor precursors, which promotes tumor precursors to spread towards the ovary and peritoneal sites.

We used two immortalized human FT cell lines, in which p53 was knocked down by shRNA. Mutant p53 or a control vector were introduced to these cell lines via a lentiviral expression system. The expression level of TrkB protein in FT cells expressing mutant p53 or a control vector was evaluated using Western blot and flow cytometry. CellTiter luminescent cell viability assays were performed to assess the effects of BDNF on the survival of FT cells with or without p53 mutation in 3D culture. Cellmate hydrogel was used as scaffold to support FT cells. And the cells migrated outside the hydrogel were quantified using CellTiter assay. Collagen I-coated beads were used in a 3D cell adhesion model to quantify the ability of FT cells to adhere to ECM and evaluate the effects of BDNF on the cell adhesion. The activation of TrkB downstream proteins was assessed using western blot.

Our data demonstrated that the protein level of TrkB was higher in FT cells expressing mutant p53 (R175H, R248W, and R273H) compared to the control FT cells. Mutant p53 significantly enhanced the survival of FT cells in the serum-free 3D culture condition in the presence of BDNF. BDNF increased the ability of FT cells to migrate outside the hydrogel, and mutant p53 further enhance their migration. Mutant p53 also accelerated the BDNF-mediated attachment of FT cells to collagen I-coated beads. Western blot results showed that the BDNF-activated phosphorylations of TrkB, AKT, ERK, PLC-gamma1 CREB were all increased in the FT cells expressing mutant p53 comparing to the control cells without mutant p53.

These results suggest the role of mutant p53 in promoting the initiation of HGSOC from FT tumor precursors. Through enhancing BDNF/TrkB signaling, mutant p53 can enhance the ability of FT tumor precursors to overcome anoikis and spread towards the ovary, peritoneum, and omentum. A better understanding of the underlying mechanisms will contribute to the development of new treatment and detection markers for HGSOC initiated from the FT.

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OMENTAL MACROPHAGES REGULATE OVARIAN CANCER METASTATIC COLONIZATION THROUGH THE CCL6-CCR1 SIGNALING AXIS

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BACKGROUND: Metastatic ovarian cancer remains an urgent clinical problem. The homing and invasion of cancer cells into the omental adipose tissue, which is the preferred site of ovarian cancer metastasis, is a critical step in disease progression. Omental adipose tissue contains adipocytes, blood vessels, and clusters of leukocytes known as milky spots. We have previously demonstrated in several mouse models that intraperitoneally injected ovarian cancer cells localize to the omentum and specifically to the milky spots. However, we found that B, T, or NK cells were not required for omental colonization. We hypothesize that macrophages within the milky spots play a crucial role in promoting ovarian cancer progression.

METHODS AND RESULTS: Depleting macrophages disrupts cancer cells' localization to the omentum. This result supports our hypothesis that macrophages play an essential role in attracting ovarian cancer cells to the omental milky spots. Correspondingly, factors secreted by omental macrophages promote ovarian cancer migration *in vitro*, in contrast to factors secreted by macrophages isolated from other peritoneal adipose tissues. In order to understand the specific mechanism(s) that regulate metastatic colonization of the omentum, we performed RNA-Seq of CD45+CD11b+F4/80+ flow-sorted macrophages from the omentum and the mesenteric fat of naïve and cancer-bearing mice. We found that omental macrophages have a distinct and dynamic gene expression pattern even when compared to a very similar cell type. The chemokine CCL6 is one of the most highly upregulated genes during metastatic colonization. The human homolog of CCL6 is CCL23 and is also expressed by human omental macrophages. Genomic inactivation of CCR1, the gene encoding receptor for CCL6 in mice and CCL23 in humans, in mouse ovarian cancer cells abolishes their ability to home to omental milky spots. Our findings establish a role for chemokine signaling in the early colonization of the omentum by ovarian cancer.

CONCLUSION: We demonstrate that omental milky spots are the preferential sites for cancer colonization of peritoneal adipose. Further, we have identified omental macrophages as the critical mediators of colonization via the CCL6/CCR1 chemokine axis. Future studies are focused on understanding the cancer cell-macrophage interactions that can be targeted therapeutically to disrupt metastatic growth and extend disease-free survival.

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MYXOMA VIRUS ENHANCES TREATMENT BENEFIT OF CHEMOTHERAPY AND DENDRITIC CELL IMMUNOTHERAPY IN OVARIAN CANCER PRECLINICAL MODEL

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Myxoma virus (MYXV) is a poxvirus with oncolytic and immunotherapeutic potential in many cancer models, including ovarian cancer (OC). Moreover, MYXV synergizes with chemotherapy including cisplatin for OC treatment. In this study, we used a p53$^{-/-}$ ID8 mouse model that closely portrays human high-grade serous OC to test MYXV virotherapy. We combined MYXV regime with cisplatin treatment and an innovative Th17-inducing dendritic cell (Th17-DC) vaccine currently being tested in clinical trials with OC patients. For DC immunotherapy, bone marrow-derived DC were pulsed with peptides from Sp17, a cancer testis antigen highly expressed in human OC and the ID8 mouse model. For MYXV virotherapy, we examined both wild-type and a replication-defective MYXV that is engineered without an essential viral immunomodulator called M062 (*M062R*-null MYXV). We found that inoculation with replication-competent MYXV improves survival when administered before cisplatin therapy. Interestingly, when the replication-defective *M062R*-null MYXV was used, improved survival was observed with virotherapy after the cisplatin treatment. We further found that *M062R*-null MYXV treatment provided long-lasting survival in combination with cisplatin and Th17-DC immunotherapy. Related studies showed that *M062R*-null MYXV infection in murine and primary human OC cells stimulated robust type I IFN expression and pro-inflammatory cytokines. Moreover, *M062R*-null MYXV activates type I IFN in macrophages and expression of inflammatory cytokines (e.g., CXCL-10 and IL-6). In conclusion, we propose that MYXV virotherapy provokes a type I interferon response and inflammatory cytokines that modulate the tumor microenvironment and enhance protective immune responses to Th17-DC vaccination for OC treatment.

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USING A DYNAMIC, LOW-SHEAR ENVIRONMENT TO MIMIC PHYSIOLOGICAL FLUID SHEAR STRESS IN OVARIAN CANCER *IN VITRO* MODELING

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Ovarian cancer is the most lethal gynecological cancer with a five-year survival rate as low as 30%. Early diagnosis could offer high chances of survival as most ovarian cancer cases are diagnosed only after metastasis has taken place. Some of the challenges in diagnosis and treatment are due to ovarian cancer's preference for the transcoelomic metastatic pathway over the more well-known hematogenic pathway, where spheroids suspended in the peritoneal cavity invade through the mesothelial layer that lines it. Spheroids for in vitro experiments have been generated for decades using common methods such as hanging drops and liquid overlay, but these models typically do not account for the fluid shear stress present in vivo, especially following the formation of ascites. One method to mimic this in vitro is to use dynamic cell culture to generate shear stress. Fluid movement parameters (rotation, shaking, etc.) can be tuned to achieve physiologically-relevant shear stress while facilitating the formation of spheroids. Dynamic culture improves the roundness and size consistency of spheroids, facilitating functional assays such as spheroid migration and mesothelial clearance. The addition of shear stress is expected to increase the metastatic behavior of spheroids as it better recreates the in vivo environment. This model also provides a platform for incorporating other in vivo elements such as co-culture, use of ascites samples, etc. This work is ultimately aimed at improving our fundamental understanding of peritoneal metastasis with new insights for determining optimal therapeutic approaches.

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INHIBITION OF TUMOR MICROENVIRONMENT CYTOKINE SIGNALING SENSITIZES OVARIAN CANCER CELLS TO ANTIESTROGEN THERAPY

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OBJECTIVE: Ovarian cancer tumor cell estrogen receptor (ER) expression alone is a poor predictor of response to antiestrogen therapy and some patients with ER-negative cancers still demonstrate clinical response to therapy, suggesting modifiers of response that can potentially be targeted to improve outcomes. We have demonstrated that IL6 and LIF cytokine signaling from carcinoma-associated mesenchymal stem cells (CA-MSC) in the tumor microenvironment promotes tumorigenesis and hypothesize that this cytokine signaling also engages in crosstalk with estrogen signaling pathways. Furthermore, we propose that blocking both cytokine and estrogen signaling will improve anti-cancer effects.

METHODS: High grade serous ovarian cancer (HGSOC) cell lines were treated with CA-MSC conditioned media or recombinant cytokines IL6 and/or LIF. Ovarian cancer cells were then assessed for (i) activation of estrogen response element (ERE)-driven luciferase reporter constructs (SABiosciences), (ii) changes in levels of reported ER target genes by qRT-PCR, and (iii) ER-alpha expression levels by immunoblotting. ER-alpha expression in CA-MSC was assessed by immunoblotting. We then assessed the effect of treating cells with ruxolitinib, the FDA-approved inhibitor of the Janus-associated kinase (JAK) protein that is downstream of IL6/LIF signaling. Tumor cells in the absence or presence of CA-MSC conditioned media were treated with ruxolitinib without or with anti-estrogen therapy. Anti-estrogens studied include the selective estrogen receptor modulator tamoxifen, the selective estrogen receptor downregulator fulvestrant, and the aromatase inhibitor letrozole. Following drug treatment, cell viability was assessed with the MTT assay and colony forming assays, and signaling cascade protein expression determined by immunoblotting. Synergy was calculated by the Chou-Talalay method using CompuSyn software.

RESULTS: IL6 and LIF induce ERE reporter construct activation in HGSOC cell lines to a similar extent as control estradiol treatment and increase the expression of known ER target genes. Additionally, IL6 and LIF increase ER-alpha levels in HGSOC cell lines as detected by immunoblotting. The treatment of HGSOC cells with both ruxolitinib and antiestrogen therapy results in a synergistic decrease in cell viability. Variable effects are noted depending on the specific antiestrogen used and the cell line studied, indicating subtle mechanistic differences between cell lines. CA-MSC express ER-alpha, suggesting antiestrogens may exert their effects at least in part through changes in stromal signaling. Further studies are underway, including characterization of effects in both primary tumor cells and animal models.

CONCLUSIONS: IL6 and LIF signaling from the tumor microenvironment promotes ovarian cancer cell estrogen signaling. The combination of inhibiting IL6/LIF signaling with ruxolitinib and antiestrogen therapy results in a synergistic decrease in ovarian cancer tumor cell viability. Given the clinical tolerability of antiestrogen therapy with a low side effect profile, strategies such as ruxolitinib treatment to sensitize tumor cells to antiestrogens are an exciting area that warrant further study.

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MECHANOTRANSDUCTION IN OVARIAN CANCERS

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INTRODUCTION: Ovarian cancers have dynamic mechanical microenvironments. Ovarian cancer cells within the tumor microenvironment experience a range of shear and compressive stimuli from both internal and external sources including ascitic fluid flow, interstitial fluid flow, hydrostatic pressure, vascular blood flow, displacement of surrounding cells, and growth induced stress, all of which contribute to mechanotransduction. Ovarian cancer progression typically comes with the development of ascites within the peritoneal cavity, which create a unique mechanical microenvironment. External dynamic stimuli have been correlated with an increase in metastasis, cancer stem cell (CSC) marker expression, chemoresistance, and proliferation in a variety of cancers however, how these shear and compressive stimuli specifically contribute to ovarian cancer progression has been overlooked. To address this gap in knowledge, individual custom built bioreactors capable of applying tunable shear stress or compressive stimulus were constructed and characterized, to investigate the impact of physiologically relevant forces on ovarian cancer cells within a 3D culture environment.

MATERIALS AND METHODS: A compression bioreactor capable of applying cyclic or static compression cycles, and a shear stress bioreactor equipped with tunable shear stress stimulus, were designed and fabricated in house. Both bioreactors utilize a 3D agarose-collagen type I interpenetrating network (IPN) hydrogel for mechanically supporting embedded cells. 20 kPa of pressure was applied for 24 hours to high grade serous OVCAR3 and OVSAHO ovarian cancer cells in either static or cyclic waveforms. Shear stress was applied to OVCAR3 and OVCAR8 cells for 24 hours via a peristaltic pump at shear stresses of 1, 5, or 11 dynes/cm2. Mechanical stimulus experienced by cells housed within the IPN gel composite was evaluated using finite element analysis (FEA). Histological stains were evaluated for morphological quantification and immunohistochemistry staining was used to evaluate proliferation and cell death. Changes in gene expression were monitored through qPCR analysis of experimental vs control groups.

RESULTS AND DISCUSSION: The IPN hydrogel was characterized using SEM imaging and rheometric testing, and the viscoelastic modulus was determined to be 10.36±0.08 kPa. FEA of the bioreactors showed the distribution of compressive or shear forces throughout the cells within the hydrogel. Under compression, OVCAR3 cells were found to have a significant increase in cellular area, aspect ratio, and a significant decrease in roundness. The cells also displayed a two-fold increase in proliferation as quantified by Ki67 expression. Cell death was found to be significantly decreased under static compression conditions when compared to either cyclic or control conditions. qRTPCR analysis revealed a greater than 2-fold increase in the expression of COX-2, BCL2, E-Cadherin, C-SRC, and CDC42. Similar results were observed with shear stress stimulation.

CONCLUSIONS: Innovative compression and shear stress bioreactors were designed and constructed to investigate the influence of physiologically relevant mechanical stimulus on ovarian cancer cells. Stimulated cells were found to exhibit cellular morphological changes, indicative of a motile phenotype. Ovarian cancer cells increased expression of a variety of genes tied to metastasis, mechanotransduction, cell cycle progression, and chemoresistance. The increased proliferation and decreased cell death indicate a cancer assistive mechanism from mechanical stimuli, and thus a potential treatment target within the activated mechanotransduction pathways. These dynamic in vitro 3D platform provide understanding of the influence of mechanical stimuli, and their influence on cellular response, a critical component of the mechanical environment in a variety of diseases and cell types.

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THE HYPOXIC TUMOR-MESOTHELIAL NICHE PROMOTES OVARIAN CANCER METASTASIS THROUGH COLLAGEN REMODELING

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High grade serous ovarian cancer (HGSOC) is a leading cause of cancer related deaths among women. The primary cause of morbidity and mortality in HGSOC patients is metastasis to organs within the peritoneal cavity. Mesothelial cells line the surface of peritoneal organs and are a key stromal cell in HGSOC metastatic niche. However, the mechanisms by which mesothelial cells promote ovarian cancer peritoneal metastasis remain largely unknown. Here we demonstrate that tumor associated mesothelial cells promote tumor invasion by increasing collagen deposition and remodeling. Mechanistically, we demonstrate the tumor-mesothelial niche is hypoxic where both tumor cells and mesothelial cells express the hypoxia inducible factors HIF-1 and HIF-2 and their downstream target gene lysyl oxidase (LOX). LOX is an enzyme that crosslinks collagen fibrils to promote collagen remodeling. In tumor-mesothelial co-culture experiments, we demonstrate that hypoxia enhances extracellular fibrillar collagen deposition by mesothelial cells. Conditioned media from hypoxic tumor-mesothelial co-cultures promotes collagen remodeling and HGSOC tumor cell invasion. Genetic inactivation of either HIF-1 and HIF-2 or LOX reduces the hypoxic induction of collagen remodeling and tumor cell invasion. Importantly, pharmacologic inhibition of HIF-1 and HIF-2 with digoxin reduces the colonization of disseminated HGSOC cells to the omentum, the preferential migration site of peritoneal cancer metastasis. Moreover, pharmacologic inhibition of LOX with BAPN (beta-aminopropionitrile) is sufficient to inhibit metastatic tumor burden and collagen remodeling at metastatic sites in preclinical models of HGSOC metastasis. These data reveal a novel role for mesothelial cells in the production of type I collagen and collagen remodeling in the HGSOC metastatic microenvironment. Furthermore, these studies demonstrate a role for the HIF/LOX signaling axis in the HGSOC tumor-mesothelial niche that can be therapeutically targeted to inhibit collagen remodeling and ovarian cancer metastatic progression.

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OVACURE - BRINGING PERSONALIZED TREATMENT TO WOMEN WITH OVARIAN CANCER

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Immunotherapy has revolutionized the treatment of many types of cancer and is now undergoing testing in ovarian cancer (OC). Several immunotherapeutic approaches are currently being developed for OC treatment; including tumor infiltrating lymphocytes based adoptive cell therapy (TIL based ACT), chimeric antigen receptor–modified T-cells (CAR T-cell), personalized vaccines and immune checkpoint blockade. Additionally, the importance of using combination of therapies and obtaining information on different group of patients (treatment responsive and unresponsive) prior to designing new approaches, has been widely identified. Although being able to choose a more targeted combination therapy can potentially be lifesaving for OC patients; identifying the successful treatment combination is challenging due to the low mutation frequency in this cancer and the variability observed patient to patient.

Our goal is to support OC patients from a synergetic point of view. OvaCure portfolio contributes to almost all the immunotherapeutic approaches mentioned above; as well as to understanding how patients respond to different treatment combinations and how this information can be used to improve patient care. OvaCure's T-cell Immune Therapy Branch consist in three parallel trials that build in and complement each other, and that aim to make a meaningful impact by moving forward TIL based ACT. Our first clinical trial is led by Prof. Svane - Denmark, and has already contributed to state-of-the-art of stablishing the medical plausibility of treating recurrent OC with TIL based ACT. The clinical Phase I/II - Immunotherapy by adoptive T-cell infusion in the event of advanced ovarian cancer (stage III/IV), will be completed in 2019 and will provide an overview of the clinical efficacy of TIL based ACT in combination with anti-CTLA-4 antibody Ipilimumab and PD-1 antibody. The second trial, clinical Phase I - Combined chemo- and adoptive T-cell therapy (ACT) as treatment for recurrent epithelial ovarian carcinoma, is led by Ass. Prof. Verdegaal - The Netherlands. Patient enrolment commenced early 2018, and the trial will provide insight on the role of interferon alfa in enhancement of T-cell survival, improvement of TILs persistence and effectiveness, and in lowering toxicity for patients. The third project in our T-cell Immune Therapy Branch is led by Prof. Coukos – Switzerland, and aims to identify the human tumour cells expressed antigenic determinants. The coming Clinical Phase I will assess the clinical efficacy of utilizing the selected tumor-specific T-cells against OC. Tumour-match: Combination of immune therapy with a next generation patient selection platform to improve treatment outcomes is also part of our portfolio. Herein, Dr. Mirza combines two breakthroughs in cancer: immune therapy with a next generation patient selection platform using RNA sequencing. This study strives to develop gene expression signatures that could guide treatment decisions, can be used to monitor disease progression and open the window to diagnostic opportunities.

OvaCure's core focus is to make a positive impression on society by supporting experimental, bold and risk-taking research; as well as, promoting awareness through public outreach. Visit our website (www.ovacure.org) for more information about our research and activities.

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A TWO-STEP TARGETING STRATEGY UTILIZING GLYCOENGINEERED MESENCHYMAL STEM CELLS FOR OVARIAN CANCER

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Current tumor targeted drug delivery systems suffer from a lack of selectivity for tumor cells. We propose a two-step tumor targeting strategy to improve the delivery of therapeutic agents to metastatic ovarian cancer. The two-step targeting approach relies on the introduction of non-natural targets (azide functional groups) in the tumor tissue, followed by the delivery of drug-loaded polymeric nanoparticles that are surface modified (with dibenzyl cyclooctyne; DBCO) and have high affinity for these synthetic targets. The objective of the present study was to determine the in vivo efficacy of two-step targeting in a clinically-realistic, patient-derived xenograft (PDX) model of ovarian cancer. The DBCO functionalized, paclitaxel (PTX)-loaded nanoparticles (16.2% w/w PTX; diameter 320 ± 10 nm; and zeta potential -11.5 ± 1.3 mV) were formulated using the FDA approved, biodegradable and biocompatible polymer, poly (DL-lactide-co-glycolide) (PLGA), by emulsion-solvent evaporation method. Glycoengineered mesenchymal stem cells (MSC-Az) were generated by culturing MSCs in N-azidoacetylmannosamine-tetraacylated supplemented media without affecting their viability or tumor homing properties. Anticancer efficacy of MSC-Az mediated two-step targeting strategy was evaluated in female athymic nude mice bearing PDX tumors. Mice with generation 2 tumors were randomly divided into four groups receiving intravenous injection of saline, PTX solution, DBCO-PTX nanoparticles, and intravenous injection of MSC-Az followed by intravenous injection of DBCO-PTX nanoparticles (MSC-Az + DBCO-PTX). We observed significantly reduced tumor growth ($p < 0.05$) and improved survival in the group receiving MSC-Az + DBCO-PTX compared to all other groups. Furthermore, immunohistochemical analysis of the tumor tissues demonstrated that the animals treated with MSC-Az + DBCO-PTX had extensive necrosis compared to other groups, suggesting the potential of MSCs for improved tumor targeting and inhibition. In summary, our results demonstrate the potential of two-step tumor targeting strategy to enhance tumor specific delivery of conventional chemotherapeutic drugs resulting in significantly improved anti–cancer efficacy.

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PKCα INDUCES TWIST1 PHOSPHORYLATION AT SERINE 144 AND PROMOTES EMT IN OVARIAN CANCER CELLS

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PURPOSE: Metastasis and carcinomatosis remains a major problem in ovarian cancer. Metastasis requires a process known as Epithelial-to-Mesenchymal Transition (EMT), where by epithelial cancer cells undergo dynamic changes to acquire a mesenchymal and invasive phenotype. Twist1 is a transcription factor with a central role in EMT. We previously showed that in epithelial ovarian cancer (EOC), Twist1 is constitutively ubiquitinated and targeted for proteasomal degradation and inhibition of Twist degradation is associated with mesenchymal phenotype and chemoresistance. Accordingly, identification of mechanisms that promote EMT by inhibiting the active degradation of Twist1 is critical for prevention of carcinomatosis. In this study, we report the identification of PKCα as a central regulator of Twist1. We identified 11 possible PKCα phosphorylation sites on Twist1. We show that Twist1 is a novel substrate of the kinase PKCα and that PKCα-induced Twist1 phosphorylation abrogates Twist1 ubiquitination leading to its stabilization and consequently EMT.

METHODS: The following cell lines were used: (1) ovarian cancer cells lines OVCAR3 and OVCA432; (2) in-house developed cultures of ovarian cancer cells, R182 and R2615; and (3) HEK293T cells. The effect of constitutively active (PKCα*CAT*), dominant negative (PKCα*DN*), and wild-type (wt-PKCα) PKCα on levels of Twist1 were determined by transient transfection using qRT-PCT and western blot analysis. Levels of phosphorylated Twist1 were measured in Twist1 immunoprecipitate-complex using anti-phospho-serine/threonine/tyrosine antibody. The phospho-deficient mutant Twist1 S144A was constructed using QuickChange Site-directed Mutagenesis Kit. PKCα was knocked-out in ovarian cancer cells using CRISPR/Cas9. EMT was induced by treatment with 10 ng/ml TGFβ1 and confirmed molecularly (i.e. loss of epithelial markers E-cadherin, Ck18, Claudin 3 and gain of mesenchymal markers Twist1, N-cadherin, and vimentin). Intra-peritoneal tumors were established by injecting 10 million ovarian cancer cells in athymic nude mice.

RESULTS: Transfection with PKCα*CAT*(active) resulted in a significant increase in Twist1 protein levels compared to PKCα*DN*(inactive) or empty vector control in EOC and HEK293T cells. This was not a transcriptional effect (no increase in Twist1 mRNA) but due to increase in the levels of phospho-serine/threonine/tyrosine on Twist1. We identified S144 as the putative PKCα phosphorylation site on Twist1 protein. Whereas wt-Twist1 was readily ubiquitinated when transfected in ovarian cancer cells and HEK293T, the phosphomimic S144D Twist1 demonstrated significantly less ubiquitination. Furthermore, we identified TGFβ1 as an activator of the endogenous PKCα-Twist1 axis in ovarian cancer cells. TGFβ1 is able to: (1) activate PKCα; (2) increase Twist1 protein levels; (3) and induce EMT (spheroid formation). Knock-out of PKCα in ovarian cancer cells abrogated TGFβ1-induced EMT *in vitro* and inhibit carcinomatosis *in vivo* in athymic nude mice model.

CONCLUSION: We demonstrate for the first time a TGFβ1-PKCα-Twist1 signaling pathway that specifically targets Twist1 protein for phosphorylation and stabilization. This is a non-classical pathway of TGFβ1 induced EMT. Moreover, we identify S144 on Twist1 as novel and direct PKCα-phosphorylation site that can control Twist1 stability. Given the pleiotropic nature of TGFβ1 signaling, the identification of PKCα as a novel target may aid in the development of better therapeutic modalities that can prevent EMT and curtail metastasis formation in ovarian cancer.

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BROMODOMAIN INHIBITION IN OVARIAN CANCER AND THE TUMOR MICROENVIRONMENT

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BACKGROUND: Ovarian cancer is the most lethal gynecologic malignancy. While women with BRCA deficient tumors show sensitivity to PARP inhibitors (PARPi), new treatment options are urgently needed for patients with PARPi-resistant tumors. An emerging strategy to improve PARPi response is combination therapy with epigenetic drugs. A recently recognized epigenetic drug target in ovarian cancer is the bromodomain and extraterminal (BET) family of proteins. BET proteins such as BRD4 promote oncogenic transcription of genes promoting cell growth, survival and DNA repair. One example is the well-established link between inflammation and cancer, nuclear factor-kappaB (NF-κB). In syngeneic mouse ovarian cancer models, M2-like pro-tumor macrophages are a prominent component of the ovarian cancer tumor microenvironment (TME). NF-κB inhibition can reduce the M2 macrophage population; however, more sustained treatment with a systemic NF-κB inhibitor leads to more ascites and reduced survival time. Thus, BET inhibitors (BETi) have the potential to induce transcriptional reprogramming in tumors and macrophages that may be beneficial or harmful depending on context.

OBJECTIVE: To determine the cellular and molecular effects of combining BETi and PARPi in mouse ovarian cancer cells and peritoneal macrophages.

METHODS: Cultured ID8 mouse ovarian cancer cells, PMJ2-PC mouse peritoneal macrophages and immortalized bone marrow-derived macrophages were treated with vehicle, the PARPi olaparib, the BETi JQ1 or the JQ1/olaparib combination for 24-72h. Sulforhodamine B (SRB) assays assessed cell growth *in vitro*. C57BL/6 mice injected intra-peritoneally (IP) with ID8 cells were treated with JQ1 (30 days, 50mg/kg by IP injection) with volume of ascites fluid, weight of harvested tumor, and number of tumor implants assessed. Markers of apoptosis (cleaved PARP or cleaved caspase-3), and DNA damage (pH2AX) were measured by immunohistochemistry or western blot. NF-κB activity was measured by luciferase assays of a NF-κB reporter plasmid. Expression of M1 (CCL3) and M2 (CD206, arginase-1) macrophage markers was measured by quantitative real-time RT-PCR (QPCR).

RESULTS: In culture, JQ1 treatment sensitized ID8 ovarian cancer cells to olaparib-induced growth inhibition, DNA damage and apoptosis. However, despite modest stimulatory effects on DNA damage and apoptosis of long-term JQ1 treatment in ID8 tumors *in vivo*, JQ1 unexpectedly increased ascites formation without reducing overall tumor burden. In macrophages, there were also contrasting effects between JQ1 treatment *in vitro* and *in vivo*. JQ1 alone or combined with olaparib reduced NF-κB activity in cultured macrophage cell lines, and increased expression of CCL3, and reduced CD206 and arginase-1 expression. In contrast, increased ascites due to JQ1 treatment *in vivo* was accompanied by a pronounced M2 macrophage shift.

CONCLUSIONS: Sustained inhibition of NF-κB activity in macrophages and potentially other cells in the ovarian TME could have overall deleterious effects on tumor progression. Our results strongly suggest that the therapeutic effects of BETi in ovarian cancer, and any therapeutic agent with the potential to alter cell function in the tumor microenvironment, need to be more thoroughly tested in the context of functional immune system before being translated to patients. This is particularly relevant since BETi are currently being tested in human patients in early clinical trials.

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SESSION 4: NOVEL THERAPEUTICS: RESPONSE AND RESISTANCE OF OVARIAN CANCER

INVITED SPEAKERS

179 **Novel combination strategies for recurrent ovarian cancer**
Ursula Matulonis, MD • *Dana-Farber Cancer Institute, Harvard Medical School*

181 **Cell Cycle Checkpoints as Therapeutic Targets**
Jung-Min Lee, MD • *National Cancer Institute*

ABSTRACT PRESENTATIONS

183 **Total Abdominal Ultra-Rapid FLASH Irradiation Demonstrates Decreased Gastrointestinal Toxicity Compared to Conventional Total Abdominal Irradiation in Mice**
Karen Levy MD • *Stanford University*

185 **Cancer Stem Cells: Distinct Seeds for Recurrent Ovarian Cancer**
Nuzhat Ahmed, PhD • *Fiona Elsey Cancer Research Institute (Australia)*

187 **A phase 2 study of TPIV200/huFR-1 (a multi-epitope folate receptor alpha vaccine) in combination with durvalumab in patients with platinum resistant ovarian cancer**
Dmitriy Zamarin,MD, PhD • *Memorial Sloan Kettering Cancer Center*

189 **Antitumor response after vaccination targeting insulin growth factor binding protein 2 in a syngeneic mouse model of ovarian cancer localized by three-dimensional in vivo optical imaging**
Denise L. Cecil, MD, PhD • *University of Washington*

191 **A single cell RNA-sequencing approach to uncovering human ovarian tumor and immune cell heterogeneity, and their response to Mullerian Inhibiting Substance using patient ascites samples**
David Pepin, PhD • *Massachussetts General Hospital, Harvard Medical School*

193 **BRCA1 intron retention generates truncated proteins that avoid BRCT mutation misfolding and promote PARP inhibitor resistance**
Neil Johnson, PhD • *Fox Chase Cancer Center*

195 **ATR inhibition targets treatment resistant ovarian cancer**
Erin George, PhD • *University of Pennsylvania*

197 **The role of NFAT3 in Ovarian Cancer Quiescence and Chemotherapy Resistance**
Alex Cole, PhD • *University of Michigan*

SESSION 4: NOVEL THERAPEUTICS: RESPONSE AND RESISTANCE OF OVARIAN CANCER (POSTER PRESENTATIONS)

198 POSTER NT–086 | SESSION I: THURSDAY
Preliminary Identification of Lactate Dehydrogenase Inhibitors towards Anticancer Drug Development
Emmanuel Adegbite, Medical Student
Bowen University (Nigeria)

199 POSTER NT–087 | SESSION II: FRIDAY
Nanoformulation of Talazoparib suppresses tumor growth and ascites in a disseminated cancer model
Paige Baldwin, BS
Northeastern University

200 POSTER NT–088 | SESSION I: THURSDAY
Prognostic Significance of NR4A1/TR3 Expression in Ovarian Cancer
Alicia Beeghly-Fadiel,PhD
Vanderbilt University Medical Center

201 POSTER NT–089 | SESSION II: FRIDAY
TRAIL-expressing oncolytic vaccinia virus combined with small-molecule drug PAC1 is a potentially effective treatment alternative for ovarian cancers
Powel Crosley, MSc
University of Alberta (Canada)

202 POSTER NT–090 | SESSION I: THURSDAY
Preclinical Activity and Safety of STRO-002, a Novel ADC Targeting Folate Receptor Alpha for Ovarian and Endometrial Cancer
Venita I. De Almeida, PhD
Sutro Biopharma

203 POSTER NT–091 | SESSION II: FRIDAY
TGF-β1-induced chemoresistance is reversed by BG-4 peptide
Vermont P Dia, PhD
The University of Tennessee

204 POSTER NT–092 | SESSION I: THURSDAY
Functional heterogeneity of acquired PARP inhibitor resistance in BRCA1-deficient cells
Anniina Farkkila, MD, PhD
Dana Farber Cancer Institute

205 POSTER NT–093 | SESSION II: FRIDAY
The N6-methylation of adenosine (m6A) in FZD10 mRNA contributes to resistance to PARP inhibitor
Takeshi Fukumoto, PhD
The Wistar Institute

206 POSTER NT–094 | SESSION I: THURSDAY
Targeted inhibition of HSP90 impairs DNA-damage response proteins and increases the sensitivity of ovarian carcinoma cells to PARP inhibitors
Rashid Gabbasov, PhD
Fox Chase Cancer Canter

207 POSTER NT–095 | SESSION II: FRIDAY
Eradication of chemoresistant metastatic ovarian cancer with IMMUNOTARGETED EPIGENOMIC NANOSURGERY (IEN)
John N. Giannios, PhD
Center of HISMolGenMed (Greece)

208 POSTER NT–096 | SESSION I: THURSDAY
Fabclavine, a secondary metabolite from Xenorhabdus budapestensis as therapy against ovarian cancer
Arvinder Kapur, PhD
University of Wisconsin

209 POSTER NT–097 | SESSION II: FRIDAY
Quantitative high-throughput screening using an organotypic model identifies two kinase inhibitors that inhibit ovarian cancer metastasis
Hilary Kenny, PhD
University of Chicago

210 POSTER NT–098 | SESSION I: THURSDAY
Window of opportunity trial: assessing the adaptive response of HGSOC to PARPi for informed combination therapies
Marilyne Labrie, PhD
Oregon Health & Science University

211 POSTER NT–099 | SESSION II: FRIDAY
Novel antibody drug conjugate (ADC) and companion diagnostics against CD248+ cancer
Chunsheng Li, PhD
University of Pennsylvania

212 POSTER NT–100 | SESSION I: THURSDAY
PARPi-induced ALDH1A1 Expression Contributes to PARPi resistance in Ovarian Cancer Cells
Lu Liu, BS
The Ohio State University

213 POSTER NT–101 | SESSION I: THURSDAY
QUADRA: A phase 2, open-label, single-arm study to evaluate single-agent niraparib treatment in patients with relapsed ovarian cancer (ROC) who have received ≥3 prior chemotherapy regimens
Kathleen Moore, MD
University of Oklahoma Health Sciences Center

214 POSTER NT–102 | SESSION II: FRIDAY
Chemically induced hypoxia promotes ovarian cancer chemoresistance
Cindy Andrea Nieto-Veloza, PhD
University of Tennessee

215 POSTER NT–103 | SESSION I: THURSDAY
Targeting ovarian tumors with OXPHOS inhibitors
Manish Patankar, PhD
University of Wisconsin-Madison

216 POSTER NT–104 | SESSION II: FRIDAY
Synergistic combination of mortalin-targeting and p53 reactivator drugs for ovarian cancer
Satish Kumar Ramraj, PhD
University of Oklahoma Health Sciences Center

217 POSTER NT–105 | SESSION I: THURSDAY
Anti-tumour effects of all-trans retinoid acid, an annexin A2-S100A10 pathway inhibitor on serous ovarian cancer
Carmela Ricciardelli, PhD
University of Adelaide (Australia)

218 POSTER NT–106 | SESSION II: FRIDAY
PIN1: a promising target for platinum-resistant high grade serous ovarian cancer
Flavio Rizzolio, PhD
Ca' Foscari (Italy)

219 POSTER NT–107 | SESSION I: THURSDAY
Chemoresistant Ovarian Cancer Stem Cells Reveal Novel Therapeutic Targets
Allison Sharrow, MD
University of California, Los Angeles

220 POSTER NT–108 | SESSION II: FRIDAY
Targeting Bromodomain Proteins as a Therapeutic Strategy for Clear Cell Carcinoma of the Ovary
Shigeta Shogo, PhD
Fred Hutchinson Cancer Research Center

221 POSTER NT–109 | SESSION I: THURSDAY
VAL-083 (dianhydrogalactitol) synergizes with PARP inhibitors in BRCA-proficient and BRCA-deficient ovarian cancer models
Anne Steino, PhD
DelMar Pharmaceuticals

222 POSTER NT–110 | SESSION I: THURSDAY
Efficacy of novel topoisomerase 1 inhibitors and their synergy with PARP inhibitors in a murine model for ovarian cancer
Ludmila Szabova, PhD
National Cancer Institute-Frederick

223 POSTER NT–111 | SESSION I: THURSDAY
The antiprogestin/antiglucocorticoid mifepristone and the HIV protease inhibitor nelfinavir cause endoplasmic reticulum stress and potentiate the toxicity of proteasome inhibition in high-grade serous epithelial ovarian cancer cells
Carlos Telleria, PhD
McGill University (Canada)

224 POSTER NT–112 | SESSION I: THURSDAY
Aptamer conjugated Nanoparticles for targeted drug delivery
Varatharasa Thiviyanathan, PhD
University of Texas Health Science Center

SESSION 4: NOVEL THERAPEUTICS: RESPONSE AND RESISTANCE OF OVARIAN CANCER (POSTER PRESENTATIONS CONTINUED)

225 POSTER NT–113 | SESSION I: THURSDAY

SC-003, an antibody-drug conjugate targeting dipeptidase 3, exhibits potent anti-tumor activity in patient-derived xenograft models of high grade serous ovarian cancer

Wolf Wiedemeyer, PhD
AbbVie-Stemcentrx

226 POSTER NT–114 | SESSION I: THURSDAY

Catalytic subunits switch drives resistance to EZH2 inhibitors in ARID1A-mutated cells

Shuai Wu, PhD
The Wistar Institute

227 POSTER NT–115 | SESSION I: THURSDAY

Pre-existence of poly-resistant cancer stem cells in high-grade ovarian cancer

Wa Xian, PhD
University of Texas

228 POSTER NT–116 | SESSION I: THURSDAY

Targeting Unfolded Protein Response for Ovarian Cancer Therapy

Yang Yang-Hartwich, PhD
Yale School of Medicine

229 POSTER NT–117 | SESSION I: THURSDAY

Inhibition of PARG, sensitizes ovarian cancer cells to PARP inhibitors and DNA damaging agents

Amber Yasmeen, PhD
McGill University (Canada)

230 POSTER NT–118 | SESSION I: THURSDAY

Sequential therapeutic targeting of ovarian cancer harboring dysfunctional BRCA1

Amber Yasmeen, PhD
McGill University (Canada)

231 POSTER NT–119 | SESSION I: THURSDAY

Target discovery of natural product inspired phyllanthusmins for treatment of high grade serous ovarian cancer

Alexandria N. Young, MD/PhD candidate
University of Illinois at Chicago College of Pharmacy

232 POSTER NT–120 | SESSION I: THURSDAY

Knockout of MTF1 results in the inhibition of EMT in ovarian cancer cells

Junming Yue, PhD
The University of Tennessee Health Science Center

233 POSTER NT–121 | SESSION I: THURSDAY

An anti-amphiregulin antibody as potential treatment for ovarian cancer

Einav Zmora, DVM
Weizmann Institute of Science (Israel)

NOVEL COMBINATION STRATEGIES FOR RECURRENT OVARIAN CANCER

Ursula Matulonis, MD

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No abstract provided.

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CELL CYCLE CHECKPOINTS AS THERAPEUTIC TARGETS

Jung-Min Lee, MD

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No abstract provided.

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TOTAL ABDOMINAL ULTRA-RAPID FLASH IRRADIATION DEMONSTRATES DECREASED GASTROINTESTINAL TOXICITY COMPARED TO CONVENTIONAL TOTAL ABDOMINAL IRRADIATION IN MICE

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OBJECTIVE: Ovarian cancer is the most common cause of gynecologic cancer-related death in the United States. The majority of patients diagnosed with ovarian cancer present with stage III or IV disease in which the tumor has disseminated beyond the ovaries and pelvic organs to the peritoneum and mesothelial lining of abdominal organs. Despite advances in ovarian cancer treatments including maximal cytoreductive surgery, chemotherapy and checkpoint-blockade immunotherapy, recurrence is common and prognosis remains poor. Ovarian cancer is a radiosensitive tumor; however, use of total abdominal irradiation (TAI) has fallen out of favor in the past 15 years due to high toxicity, particularly of the gastrointestinal (GI) tract. In prior studies, ultra-rapid FLASH radiation spares normal tissues, such as the lung and skin, from toxic effects of radiation. This suggests that FLASH may be an effective strategy to reduce complications of radiotherapy while maintaining antitumor control. We developed a FLASH irradiation system for mice using a linear accelerator that generates 16 MeV electrons at a high beam current and delivers large doses of radiation in a single beam in <500ms. Conventional radiotherapy delivers a dose-rate of 3-4 Gy/minute, while FLASH radiotherapy delivers a dose-rate of >40 Gy/second. Our objective is to develop a method for delivering TAI using FLASH and assess toxicity.

METHODS: Female C57BL/6 mice received TAI using FLASH and conventional (CONV) radiation in increasing doses: 8.5 Gy, 10.5 Gy and 12 Gy. Normal tissue toxicity was determined by measuring total body weights, stool counts, histological analysis, and survival.

RESULTS: Seven cohorts of mice were analyzed: five unirradiated controls, eight received 8.5Gy, five received 10.5Gy and five received 12 Gy of either TAI-FLASH or TAI-CONV. Stool counts were unchanged from controls in TAI-FLASH mice 5 days post-irradiation at all doses. In the TAI-CONV cohort, a 50% stool quantity decrease was noted after 8.5Gy and a 63% stool quantity decrease was noted after 12Gy at 5 days post-TAI. Histological analysis of the duodenum post-irradiation demonstrated that TAI-FLASH has a protective effect on the mucosal architecture. Weights remained unchanged across all groups. The survival analysis was most notable for all of the TAI-CONV mice having died by day 9 whereas all of the TAI-FLASH mice survived.

CONCLUSIONS: These data demonstrate FLASH protects against death from TAI and improves the epithelial integrity of the lower GI tract following TAI compared to conventional radiation in a preclinical model. Our discovery that FLASH is a safe strategy to deliver effective doses of total abdominal radiation potentially identifies a new opportunity to utilize TAI-FLASH for treatment of ovarian peritoneal metastases.

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CANCER STEM CELLS: DISTINCT SEEDS FOR RECURRENT OVARIAN CANCER

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PURPOSE: The treatment of ovarian cancer (OC) with chemotherapy leaves resistant cancer cells which in a short time re-grow as recurrent cancer. A diverse array of resistance mechanisms for chemotherapy has been described but none have proven as viable targets in a clinical setting. Cancer stem cells (CSCs) are increasingly accepted as the putative mediators of chemoresistance and relapse of cancer. This study aimed to understand the molecular mechanisms involved with chemoresistance and recurrence by investigating the proteomic profile of ascites-derived tumor cells obtained from OC patients prior to (chemonaive, CN) and after chemotherapy treatments (recurrent, CR).

METHODS: Ascites collected from CN and CR OC patients diagnosed with advanced-stage serous OC were cultured using a novel in vitro method to obtain a distinct population of epithelial tumor cells. Flow cytometry and immunofluorescence were used to characterize the tumor population. High-resolution label-free quantitative proteomic profiling was used to define significantly differentially expressed proteins between CN and CR tumor cells. KEGG and DAVID software's were used to determine pathways associated with CR cells. The mechanisms of survival of cisplatin and paclitaxel-treated OC cancer cells were determined in vitro by mRNA analysis and in vivo mouse xenograft models by immunohistochemistry.

RESULTS: Proteomic profiling of CN and CR tumor cells showed significant differences in proteins encoding for CSC, immune surveillance, DNA repair mechanisms, cytoskeleton rearrangement, cell-cell adhesion, cell cycle pathways, cellular transport, and proteins involved with glycine/proline/arginine synthesis in tumor cells isolated from CR relative to CN patients. Pathway analyses revealed enrichment of chemoresistance markers, energy metabolism, DNA repair mechanisms and immune surveillance pathways in recurrent CR tumor cells. Validation of these pathways in OC cell lines and nude mice treated with chemotherapy demonstrated increased expression of CSC and chemoresistant markers, enhancement in glycolytic pathway and oxidative phosphorylation, suppression of immune surveillance in chemotherapy treated cell lines and recurrent mice xenografts compared to untreated control.

CONCLUSION: These findings unravel some of the molecular mechanisms by which chemoresistance and relapse occur in OC patients' post-chemotherapy treatment; and may be important in designing novel therapeutic options for advanced-stage OC patients.

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A PHASE 2 STUDY OF TPIV200/HUFR-1 (A MULTI-EPITOPE FOLATE RECEPTOR ALPHA VACCINE) IN COMBINATION WITH DURVALUMAB IN PATIENTS WITH PLATINUM RESISTANT OVARIAN CANCER

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INTRODUCTION: Therapy with single-agent immune checkpoint inhibitors in ovarian cancer (OC) to date has demonstrated marginal benefit, calling for rational combinations. Vaccination against tumor-associated antigens (TAA's) is a potential strategy to increase therapeutic efficacy by enhancing immunogenicity.

Folate receptor alpha (FR**α**) is overexpressed in the majority of ovarian cancers (OC) and presents a compelling antigenic target for immunotherapy. TPIV200 is a GM-CSF-adjuvanted multi-epitope peptide anti-FR**α** vaccine targeting the most highly-antigenic moieties of FR**α**, which in recent phase I studies elicited durable immune response in over 90% of patients with ovarian and breast cancer. The current phase II study sought to examine whether a combination of TPIV200 with PD-L1 inhibitor durvalumab would result in enhanced anti-tumor immunity and therapeutic efficacy in patients with advanced platinum-resistant OC.

METHODS: Twenty-seven patients with platinum resistant or refractory OC were enrolled over a 10-month period. Treatment was administered in 28-day cycles. Patients were treated with TPIV200 and GM-CSF on day 1 for 6 cycles and durvalumab on days 1 and 15 at 10mg/kg for 12 cycles. Radiologic assessments were conducted every 12 weeks. Treatment was continued until completion, evidence of clinical or radiologic progression, intolerance, or withdrawal. Exploratory correlative endpoints included tissue microenvironment analyses, including expression of PD-L1 and FR**α**, and peripheral vaccine-specific immune responses.

RESULTS: The study enrolled 27 women with advanced OC. Median age at enrollment was 64 (42-76). Median number of prior lines of therapy was 3 (range 1-8). Of these patients, 85% (23) had high grade serous OC. There were no objective responses seen on the study. PFS rate at 24 weeks was 22%, with median PFS of 12 weeks. Six patients remained on treatment beyond 24 weeks. The majority of patients post-progression went on to receive subsequent standard therapy with durable clinical benefit. At the median follow up of 15.4 months, median OS was not reached.

CONCLUSIONS: TPIV200/huFR-1 and durvalumab can be safely combined in heavily-pretreated patients with platinum-resistant OC. A subset of patients exhibited durable disease stabilization. Post-immunotherapy follow up was suggestive of improved clinical benefit from standard therapies, creating a rationale for exploration of these agents in combination with chemotherapy.

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ANTITUMOR RESPONSE AFTER VACCINATION TARGETING INSULIN GROWTH FACTOR BINDING PROTEIN 2 IN A SYNGENEIC MOUSE MODEL OF OVARIAN CANCER LOCALIZED BY THREE-DIMENSIONAL IN VIVO OPTICAL IMAGING

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INTRODUCTION: Ovarian cancer often disseminates as miliary implants throughout the peritoneal cavity presenting challenges for tumor quantitation in both the clinical and pre-clinical settings. The distribution of tumor implants shows affinity for areas such as the omentum and lymph nodes, which may be included in a cytoreductive surgery. Immunotherapies are being studied for ovarian cancer due to the high rate of relapse and resistance to available treatments in the recurrent setting. Pre-clinical models for ovarian cancer often do not allow meaningful assessment of novel immune-based therapies. We hypothesized vaccination targeting insulin growth factor binding protein 2 (IGFBP-2) will result in antitumor responses that can be both quantitated and localized spatially with optical imaging to identify tumor implants most resistant to vaccination.

METHODS: C57BL/6/BrdCrHsd-Tyrc mice received 4 immunizations with IGFBP-2 peptides corresponding to epitopes known to favor antitumor immunity with Freund's adjuvant (treated) or with adjuvant alone (control). 5 x 106 cells of a syngeneic mouse model expressing codon-optimized firefly luciferase (luc2) known to replicate the immune microenvironment of human ovarian cancers were injected intraperitoneally. After two weeks mice were anesthetized prior to injection of D-luciferin and during imaging via inhalation of isoflurane. Mice were placed in conforming animal molds (BCAM: InVivo Analytics, Inc.) and multi-view multi-spectral image acquired with the InVivoPLOT mirror-gantry. Bioluminescent tomographic (BLt) images were reconstructed using InVivoAX (InVivo Analytics, Inc.) cloud-based software and aligned to the organ probability map (OPM), a type of statistical mouse atlas.

RESULTS: Surface light intensity showed a 5.41 x 109 photons/second/mouse in control compared to 1.32 x 109 photons/second/mouse in IGFBP-2 vaccinated when measured in two dimensions. Multi-spectral tomographic reconstruction was able to show a greater than 2-fold decrease in the spatial light distribution of bioluminescent metastasis consistent with the surface light intensity distribution of the BLt image. BLt images mapped to the OPM discriminate the miliary distribution of metastases in the peritoneum. Individual metastasis in the control were shown to have a greater relative light density than the individual metastasis in the IGFBP-2 vaccinated group.

CONCLUSIONS: In vivo anatomic localization of antitumor activity in a disseminated ovarian cancer mouse model can be achieved using three-dimensional optical imaging. Local differences in antitumor response to immunotherapies may be exploited to optimize their application to ovarian cancer. Identifying where tumor implants resistant to immunotherapies are sequestered may improve pre-clinical testing of immunotherapy combinations and optimally integrate them into current standard therapies.

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A SINGLE CELL RNA-SEQUENCING APPROACH TO UNCOVERING HUMAN OVARIAN TUMOR AND IMMUNE CELL HETEROGENEITY, AND THEIR RESPONSE TO MULLERIAN INHIBITING SUBSTANCE USING PATIENT ASCITES SAMPLES

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Mullerian inhibiting substance (MIS) has been shown to inhibit the growth of a stem-like population of ovarian cancer cells both in vivo and in vitro. We have previously shown that its receptor, MISRII, is expressed in a majority of ovarian serous adenocarcinomas. However the determinants of response and the pathways elicited by MIS treatment have been difficult to elucidate due the heterogeneity of tumors both across the patient population and within each sample. To address this complexity we have developed an assay in which patient cancer cells and immune cells are isolated from ascites of recurrent chemoresistant ovarian cancer and incubated in cleared supernatant from autologous ascites for 24h in presence of drug (MIS 10ug/ml) or vehicle control (N=3). This protocol allows for short-term culture in conditions nearly identical to the peritoneal environment. A parallel experiment was conducted using matching pure primary cancer cell lines (derived from the ascites samples) treated in media (MIS 10ug/ml or vehicle for 24h), but lacking immune cells and soluble factors otherwise present in ascites. The effect of treatment on cell sates, and the interaction between immune cells and cancer cells was interrogated by clustering analysis of single cell RNA sequencing (inDROP). We uncovered a high degree of heterogeneity of expression of known and novel markers related to epithelial-mesenchymal states and stemness markers, both across patients and within patient samples, suggesting dynamically coexisting cancer cell states and hierarchies that are patient-specific. These markers were validated in vitro across a larger cohort of primary patient cells lines (N=12) grown as tumor spheroids (with and without MIS 10ug/ml for 24h). Additionally, we have identified a varying abundance of immune cell types (Macrophages, dendritic cells, NK cells, T cells, B cells), and an unexpected effect of MIS on gene expression in both macrophage and cancer cells. The knowledge gained on the response rate in patients, and the biomarkers identified in this study will be fundamental for the successful translation of MIS to the clinic, and further our understanding of the role of non-cell autonomous inhibition of cancer cells by immune components and patient heterogeneity.

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BRCA1 INTRON RETENTION GENERATES TRUNCATED PROTEINS THAT AVOID BRCT MUTATION MISFOLDING AND PROMOTE PARP INHIBITOR RESISTANCE

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INTRODUCTION: BRCA1 BRCT domain mutations result in protein structural defects, and consequently misfolded proteins are subject to proteasomal degradation. Loss of BRCA1 protein activity results in sensitivity to PARP inhibitor (PARPi) therapy.

EXPERIMENTAL PROCEDURES: In this study, we examined PARPi resistance using the SNU-251 human endometrial ovarian cancer cell line. SNU-251 cells harbor a nonsense mutation c.5444G>A (E1815X) in BRCA1 exon 23; that disrupts BRCT domain folding. Cells were cultured in the presence of increasing concentrations of the PARPi rucaparib until resistant cells emerged. RT-PCR, Western blotting and mass spectrometry were used to measure BRCA1 mRNA and protein levels.

RESULTS: PARPi resistant cell lines all demonstrated elevated BRCA1 protein levels that could be detected with N- but not C-terminal specific antibodies. Notably, the gel migration and molecular weight of BRCA1 was different from the mutation-induced stop codon expected size. Mass spectrometric analyses identified BRCA1 peptides encoded by exons 2-15; however, despite harboring a mutation in exon 23, no peptides encoded by exons 16-24 were present in SNU-251 cells. RT-PCR analyses showed that SNU-251 cells translated protein from exon 15 and into intron 15, terminating at an intron 15 generated stop codon. We overexpressed BRCA1 cDNA that harbored stop codons located in either the BRCT domain or in intron 15. BRCT mutation containing constructs had undetectable protein levels due to protein misfolding, and cells were highly PARPi sensitive. In contrast, cells expressing the BRCA1 intron 15 encoded stop codon had robust protein expression, demonstrated RAD51 foci and chemotherapy resistance in vitro and in vivo.

CONCLUSIONS: In summary, we discovered that BRCA1 intron translation generates new stop codons resulting in loss of the entire BRCT domain. BRCTless proteins avoid mutant protein folding problems and promote residual DNA repair and chemotherapy resistance.

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NOTES

ATR INHIBITION TARGETS TREATMENT RESISTANT OVARIAN CANCER

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Introduction: Although new treatments for ovarian cancer have been developed with the introduction of Poly (ADP-ribose) polymerase inhibitors (PARPi) to standard of care platinum chemotherapy, the majority of tumors acquire drug resistance. Strategies to circumvent PARPi and platinum resistance are needed and our study addresses this gap. We hypothesize that PARPi resistant tumors are dependent on ATR/CHK1 for DNA repair, and by targeting ATR in combination with PARPi would be more effective in eradicating tumor growth.

Experimental Procedures: Drug resistant cells were developed by long term treatment of PEO1 (BRCA2 mutant) and JHOS4 (BRCA1 mutant) ovarian cancer cells with PARPi (AZD2281) or Carboplatin. Cell viability, colony formation, cell cycle and apoptosis were evaluated. Homologous recombination was evaluated by Rad51 foci immuno staining. Replication fork asymmetry was counted by DNA combing assay. PARPi resistant orthotopic PDX models (germline BRCA1/ 2 mutant) were developed by long term treatment with PARPi. PDX developed from a platinum-resistant HGSOC patient with ***CCNE1*** amplification was also tested to evaluate PARPi +/- ATRi. Whole genome sequencing, Reverse-Phase Protein Array Analysis (RPPA) and IHC were performed on cells and xenografts to evaluate for biomarkers of response.

Results: ATR/CHK1 pathway was constitutively activated in treatment resistant cell lines. Monotherapy with ATRi alone in vitro modestly induced cell death and DNA damage in PARPi and platinum resistant cell lines. Treatment with ATRi in combination with PARPi is synergistic in reducing survival of these drug resistant cells. Combination treatment was more effective in targeting cell cycle mediators, and promoting apoptosis. HR was restored in PARPi and platinum treatment resistant PEO1 cells. ATRi treatment suppressed restored HR and increased stalled replication fork. PARPi and ATRi combination treatment was synergistic in causing tumor regression in PARPi/Carboplatin resistant PDX models.

Conclusions: Our work suggests treatment resistant cells are more dependent on ATR/CHK1 pathway and ATR is a promising target for augmenting PARPi and platinum response in treatment resistant HGSOCs.

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THE ROLE OF NFAT3 IN OVARIAN CANCER QUIESCENCE AND CHEMOTHERAPY RESISTANCE

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Despite the fact that the majority of patients with ovarian cancer will have a complete clinical remission with combined surgical/chemotherapeutic approaches, Epithelial Ovarian Cancer (EOC) is the fifth most common cause of cancer-related death in American women, with the third highest mortality:incidence ratio. The majority of deaths from ovarian cancer are related to disease recurrence and the subsequent development of ultimately fatal chemotherapy-resistant disease. Elucidating mechanisms of chemoresistance in ovarian cancer cells may identify critical therapeutic targets to prevent or treat relapsed ovarian cancer. One feature that might contribute to the chemotherapy resistance is quiescence.

Quiescence describes a reversible non-proliferative cell state. As such, quiescent cells have reduced susceptibility to chemotherapeutics which target rapidly proliferating cells. Indeed, quiescence is a known mechanism of chemotherapy resistance of normal stem cells. In normal stem cells quiescence is mediated by the NFAT family of transcription factors. Unfortunately, very little is known about the factors that mediate ovarian cancer cell quiescence. Using qRT-PCR, we evaluated the expression of NFAT family members in ovarian cancer cells. We found NFAT3 to be enriched in patient ovarian cancer stem-like cells (CSLC) compared to bulk cancer cells. Treatment of EOC cells with cisplatin chemotherapy resulted in a nuclear translocation of NFAT3 and increase in NFAT3 transcriptional activity. To directly investigate the functional role of NFAT3 in ovarian cancer, we created ovarian cancer cell lines expressing constitutively nuclear/active (cNFAT3) or inducible constitutively active (IcNFAT3) NFAT3. We found that while cNFAT3 expression in ovarian cancer cells did not impact cell viability, senescence or apoptosis, cNFAT3 expression profoundly restricted ovarian cancer cell proliferation, with a 3-fold decreased cell division rates, and cellular arrest in the G0 phase of the cell cycle. Consistent with a quiescent cell phenotype, this was associated with a 10% decrease in cell size, and a 25% decrease in total cellular RNA. Induction of cNFAT3 expression in vivo resulted in tumor growth arrest. This growth arrest resulted in chemotherapy resistance such that cNFAT3 tumors treated with high dose of chemotherapy rapidly expanded after cNFAT3 inactivation. Furthermore, NFAT inhibition with the peptide inhibitor VIVIT enhance chemotherapy response in vitro and in vivo.

Taken together, our data suggests NFAT3 drives a quiescent state in CSLC and thereby mediates chemoresistance. Thus NFAT3 represents a therapeutic target to both overcome chemotherapy resistance in quiescent cancer cells and to restrict the growth of therapy-resistant disease.

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PRELIMINARY IDENTIFICATION OF LACTATE DEHYDROGENASE INHIBITORS TOWARDS ANTICANCER DRUG DEVELOPMENT

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In tumor cells undergoing rapid cellular division, significantly increased quantities of lactic acid are produced in an anaerobic respiration via the Cori cycle. While this produces a lower of the pH in the local environment, the hepatic conversion of the generated lactate into glucose places a huge energy demand on the body which makes the patient weaker and results in cancer cachexia in all ovarian malignancies. Thus, instead of producing acetyl CoA, the dividing tumorous cells synthesize lactic acid catalyzed by the enzyme lactate dehydrogenase (LDH). LDH's involvement in tumor initiation and metabolism primarily involves a state of fermentative glycolysis catalyzed by the A form of the enzyme which allows tumorous cells convert the majority of their glucose stores into lactate even under anaerobic conditions which invariably shifts the utilization of glucose metabolites from simple energy production to an active promotion of accelerated cell growth and replication. This makes LDH a vital target for malignant ovarian drug development. And in the present work a combination of virtual screening, database scouting and biophysical analysis of binding site properties have been employed in analysis the interaction of about 30,000 compounds with LDH. Using a synthetic NADH inhibitor, as a reference, only four compounds were found to demonstrate stronger binding features than the inhibitor. This preliminary in silicon screening represents the foundational effort in a ovarian cancer drug discovery project aimed at generating specific inhibitors of LDH for use in ovarian cancer therapeutics.

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NANOFORMULATION OF TALAZOPARIB SUPPRESSES TUMOR GROWTH AND ASCITES IN A DISSEMINATED CANCER MODEL

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Talazoparib, a potent PARP inhibitor (PARPi), induces synthetic lethality in BRCA-deficient cancers making it an attractive candidate for ovarian cancer treatment. However, its potency lends itself to side effects associated more closely with traditional chemotherapeutics than other clinically approved PARPi's. We sought to formulate Talazoparib in a nanoparticle delivery system such that the drug could be administered intraperitoneally, localizing the entire dose at the disease site, to increase therapeutic efficacy and minimize toxicity. NanoTalazoparib was formulated and characterized and found to have a mean diameter of 70 nm and a neutral surface charge. Talazoparib and NanoTalazoparib were tested on a panel of murine tubal and human HGSOC cell lines and dose response compared to the first clinically approved PARPi, Olaparib. Dose response data indicated all cell lines were more sensitive to Talazoparib and NanoTalazoparib than Olaparib and all lines showed the same sensitivity to nanoformulations as free drugs. The human cell lines had various BRCA mutations and deletions, as well as a homologous recombination proficient (HRP) line, however, the HRP line was more sensitive to treatment than some HRD lines. Therapeutic efficacy was tested in vivo in a murine cancer model that mimics disseminated peritoneal disease. NanoTalazoparib 3X weekly for 8 weeks did not shrink tumors but resulted in tumor growth inhibition of 64% while an equivalent dose of oral Talazoparib only resulted in 34% growth inhibition. NanoTalazoparib suppressed the average volume of ascites at the study endpoint by 3.45 times more than oral Talazoparib. H&E staining of the tissues indicated no significant toxicity to the organs of the mononuclear phagocyte system. These results indicate that NanoTalazoparib can be used to localize PARPi therapy to the peritoneal cavity for disseminated late stage ovarian cancer treatment. Our data suggests that NanoTalazoparib could be utilized to delay the formation of tumor ascites for women with HR-deficient disease. While NanoTalazoparib did not effectively treat the disseminated disease at this dose, it may have clinical utility, either in combination with other therapies or as a maintenance therapy. Preclinical data indicates PARP inhibitors potentiate damage when combined with other cytotoxic treatments, however, in the clinic this has resulted in enhanced toxicity, forcing dose reduction and delay. The IP administration of NanoTalazoparib may provide a route to bypass some of the toxicities that have plagued combination treatments.

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PROGNOSTIC SIGNIFICANCE OF NR4A1/TR3 EXPRESSION IN OVARIAN CANCER

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BACKGROUND: A key clinical challenge in ovarian cancer is identifying new strategies to treat patients who do not respond to poly ADP-ribose polymerase (PARP) inhibitor (PARPi) therapy. We previously linked the nuclear orphan receptor NR4A1/TR3 to pro-growth and pro-survival effects in ovarian cancer cells. However, it is unknown whether inhibiting NR4A1 function has therapeutic effects alone or in combination with PARPi in vitro. Moreover, the prognostic value of NR4A1 expression in patient tumors remains ill-defined, as two prior reports had contradictory findings for associations with ovarian cancer survival.

OBJECTIVE: We undertook this study to test the therapeutic potential of inhibiting NR4A1 in ovarian cancer cells, and to clarify the prognostic value of NR4A1 expression in patient tumors.

METHODS: In a panel of established ovarian cancer cell lines (OVCAR-3, OVCAR-4, SKOV-3), we inhibited NR4A1 using the chemical antagonist, 1,1-Bis(3'-indolyl)-1-(p-hydroxyphenyl) methane (C-DIM) and siRNA targeting NR4A1 (siNR4A1). Effects of C-DIM on cell growth, alone and in combination with the PARPi, olaparib and rucaparib, were assessed in sulforhodamine B (SRB) in vitro assays. Markers of apoptosis (cleaved PARP, cleaved caspase-3) and proliferation (Ki67, PCNA, p21) were measured by western blot or immunohistochemistry (IHC). In ovarian tumors, NR4A1 was measured by IHC in 203 clinically annotated formalin-fixed paraffin-embedded (FFPE) tissue samples from the Vanderbilt University Medical Center (VUMC) Tissue Repository for Ovarian Cancer (TROC). Staining intensity (1: weak; 2: moderate; 3: strong) and percent of positive nuclei (0-100) were multiplied to yield an H score for NR4A1 expression. Associations with progression-free survival (PFS) and overall survival (OS) were quantified by Hazards Ratios (HR) and 95% Confidence Intervals (CI) from proportional hazards regression.

RESULTS: In ovarian cancer cell lines, C-DIM induced concentration-dependent decreases in cell growth and markers of proliferation, and stimulated apoptosis. These effects were mimicked in cells transfected with siNR4A1 compared to a non-targeting siRNA-transfected control. In combination with PARPi, C-DIM induced synergistic growth inhibition and apoptosis in vitro. In tumors, NR4A1 expression lower than the median (H score <153.6) was more common among later stage, higher grade, serous tumors with suboptimal cytoreduction or platinum resistant disease. Higher NR4A1 expression was associated with better OS (HR: 0.52, 95% CI: 0.37-0.74) in unadjusted analyses. However, after adjustment for important prognostic covariates, including age, stage, grade, and histologic subtype, higher NR4A1 was associated with significantly shorter PFS (HR: 2.35, 95% CI: 1.29-4.28).

CONCLUSIONS: Our current results reconcile the discrepancy between prior NR4A1 reports, as associations differed due to confounding by clinical covariates. Shorter survival among cases with higher NR4A1 expression is supported by experimental evidence showing reduced ovarian cancer cell growth and increased apoptosis following NR4A1 inhibition, both alone and when combined with a PARPi. Together, our findings support further development of NR4A1 inhibition as a novel therapeutic approach that could improve response to PARPi therapy among ovarian cancer patients with chemoresistant disease.

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TRAIL-EXPRESSING ONCOLYTIC VACCINIA VIRUS COMBINED WITH SMALL-MOLECULE DRUG PAC1 IS A POTENTIALLY EFFECTIVE TREATMENT ALTERNATIVE FOR OVARIAN CANCERS

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INTRODUCTION: Procaspase activating compound-1 (PAC1) is a small-molecule drug shown ***in vitro*** to sequester inhibitory zinc ions from caspase-3. TNF-related apoptosis-inducing ligand (TRAIL) is a pro-apoptotic ligand that binds membrane-bound death receptors, triggering the extrinsic apoptotic pathway. Both agents display low toxicity in humans. Vaccinia virus (VACV) is a double-stranded DNA virus that has shown therapeutic efficacy in clinical trials and has an established safety profile in humans due to its use as the smallpox vaccine.

First-line standard of care for ovarian cancer is combination taxane and carboplatin, which has significant potential toxicity and 70% of women who receive it suffer relapse. Clinical trials involving TRAIL, both alone and combined with other drugs, have shown that while it is well-tolerated it is ineffective partly due to insufficient dosing at the tumour site.

In an effort to uncover a safer, more effective therapeutic for ovarian cancer we report here on the successful construction of a recombinant oncolytic vaccinia virus expressing TRAIL ($VACV^{TRAIL}$). Secretion of TRAIL by VACV-infected cancer cells will result in localized administration of TRAIL at higher dosages and minimize potential side-effects. We posit that treatment with PAC1 and $VACV^{TRAIL}$ represents a potentially safe, effective treatment for ovarian cancers.

RESULTS: Testing in cell line models of granulosa cell tumour (GCT) have shown that recombinant human (rh)TRAIL is effective in combination with PAC1. Dose-response assays established that combination of PAC1 (20 μM) with rhTRAIL (10 ng/mL) **dramatically reduced viability of cancer cells while being substantially less toxic to normal cells**. Replication of those assays on patient-derived primary and recurrent GCT cells confirmed PAC1 combined with rhTRAIL was dramatically more cytotoxic than treatment with rhTRAIL or PAC1 alone.

To optimize delivery of TRAIL to tumour cells, we constructed a recombinant $VACV^{TRAIL}$ virus that secretes TRAIL in the range of 70–80 ng/mL. Dose-response curves showed $VACV^{TRAIL}$ to be strongly cytotoxic with an ED_{50} of 0.1 plaque forming unit (PFU) per cell. Comparing toxicity of $VACV^{TRAIL}$ to a non-TRAIL-expressing VACV established that secretion of TRAIL is the basis for $VACV^{TRAIL}$ superiority in killing GCT cells, and supernatant collected from infected cells is more effective at reducing cell viability when combined with PAC1 than is rhTRAIL combined with PAC1.

CONCLUSION: We have successfully constructed a TRAIL-expressing oncolytic VACV which produces effective levels of active TRAIL from infected cells. Results *in vitro* suggest combining PAC1 with oncolytic $VACV^{TRAIL}$ will allow localized delivery of TRAIL resulting in a safe, synergistic, self-amplifying therapy.

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PRECLINICAL ACTIVITY AND SAFETY OF STRO-002, A NOVEL ADC TARGETING FOLATE RECEPTOR ALPHA FOR OVARIAN AND ENDOMETRIAL CANCER

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OBJECTIVES: Folate receptor alpha (FolRα) is a cell-surface glycoprotein, highly expressed in ovarian and endometrial adenocarcinoma, and thus a promising target for cancer therapy using antibody drug conjugates (ADCs). Most ADCs currently in development are generated by random attachment of the cytotoxic payload to the antibody and result in a heterogeneous mixture, comprised of many different forms that are likely to vary in stability and activity, and therefore may be suboptimal therapeutic agents. We have employed an *E. coli* cell-free expression system (XpressCF™) and site-specific conjugation technology, to generate STRO-002, a novel homogenous FolRα-targeting ADC. STRO-002 was optimized by selection of the antibody, drug-linker, conjugation site and drug-antibody ratio (DAR) that conferred the best pharmacological properties. We have conducted preclinical studies to evaluate the stability of STRO-002 and characterize the pharmacological properties of the cytotoxic metabolite SC209. In vitro cytotoxicity assays and in vivo efficacy studies were conducted to evaluate the activity of STRO-002 in multiple ovarian cancer cell lines and xenografts. IND enabling toxicology studies were conducted to determine the safety profiles for STRO-002 and its metabolite SC209 in cynomolgous monkeys and rats, respectively.

RESULTS: Based on optimization studies, the anti-FolRα human IgG1 antibody (H01/SP8166) conjugated to a proprietary cleavable drug-linker (SC239) was selected for the lead ADC STRO-002. SC239 contains a tubulin-targeting 3-aminophenyl hemiasterlin warhead, SC209, which has potent cytotoxic activity. Based on most favorable anti-tumor activity, positions 180 and 404 on each heavy chain were selected for conjugation of SC239 to SP8166 to yield an ADC with DAR of ~ 4.

The drug-linkage in STRO-002 is highly stable and the released warhead, SC209, is a very weak substrate for cellular drug-resistance efflux pumps and is cleared rapidly from plasma. STRO-002 has potent but highly specific cytotoxic activity (0.1-3 nM) on multiple FolRα-positive ovarian cancer cell lines in vitro and anti-tumor efficacy in ovarian xenograft models. STRO-002 exhibits dose-dependent tumor growth inhibition in Igrov-1 tumor xenografts at a single dose and complete regression is achieved in Igrov-1 and OVCAR-3 tumors with a single dose at 10 and 5 mg/kg, respectively. In addition, administration of STRO-002 in combination with carboplatin confers added benefit in efficacy in Igrov-1 tumors. Toxicology studies show favorable safety profiles for STRO-002 and SC209. The main toxicity finding in monkeys dosed up to 9 mg/kg consists of reversible hematopoietic/lymphoid tissue toxicity, which is considered antigen-independent and is consistent with the anti-proliferative effects of SC209 observed in single-dose toxicology studies in rats. No evidence of ocular toxicity due to SC209 were observed in either species.

CONCLUSIONS: STRO-002 is a highly specific FolRα targeting ADC with minimal drug moiety release in circulation and the potential for an improved safety and activity profile, and a reduced risk of tumor drug resistance. Our data supports the advancement of STRO-002 to the clinic as a potential treatment of FolRα expressing malignancies such as ovarian cancer.

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TGF-β1-INDUCED CHEMORESISTANCE IS REVERSED BY BG-4 PEPTIDE

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Transforming growth factor-β (TGF-β) superfamily plays a significant role in ovarian physiology but dysregulated TGF-β signaling is associated with promotion of ovarian carcinogenesis. TGF-β signaling in ovarian cancer favors cancer invasion and metastasis and controls ovarian cancer cell proliferation. The objective of this study is to determine the ability of a food-derived peptide, called BG-4, to suppress TGF-β1 signaling in ovarian cancer cells. BG-4 is a novel bioactive peptide isolated from the seeds of *Momordica charantia* with demonstrated anticancer and strong trypsin inhibitory properties. Different ovarian cancer cells (A2780-1A9, BR and PEO4) were exposed to 10 ng/mL TGF-β1 with or without non-cytotoxic dose of BG-4 (100 µg/mL) and response to chemotherapeutic reagents paclitaxel and cisplatin was evaluated. In addition, markers of epithelial-to-mesenchymal transition (EMT) and apoptosis were evaluated using western blot and immunofluorescence microscopy. TGF-β1 treatment resulted in cisplatin resistance in three cell lines as evidenced by increase in ED50 values from 42.9 µM to 88.5 µM, 53.7 µM to 108.3 µM and 44.8 µM to 67.3 µM for BR, PEO4 and A2780-1A9 cells, respectively. In the presence of BG-4, ED50 values of 35.6 µM, 56.4 µM and 46.6 µM were obtained for BR, PEO4 and A2780-1A9 cells, respectively. These values are similar to non-TGF-β1-treated cells indicating the ability of BG-4 to reverse TGF-β1-induced chemoresistance in ovarian cancer cell lines. Mechanistic studies showed that TGF-β1 signaling induces EMT in ovarian cancer cells as shown by differential expression of epithelial marker E-cadherin and mesenchymal markers N-cadherin, vimentin and transcription factors Snail, Slug and Twist1. In addition, the expression of apoptosis markers were affected by TGF-β1 signaling. On the other hand, treatment of TGF-β1 in the presence of 100 µg/mL BG-4 led to reversal of the EMT process. These results showed that the ability of BG-4 to reverse TGF-β1-induced chemoresistance in ovarian cancer cell lines may be associated with inhibition of the EMT process.

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FUNCTIONAL HETEROGENEITY OF ACQUIRED PARP INHIBITOR RESISTANCE IN BRCA1-DEFICIENT CELLS

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INTRODUCTION: More than 50% of high-grade serous ovarian cancers (HGSOC) are deficient in Homologous Recombination (HR) DNA repair, predominantly due to inactivation of *BRCA1* or *BRCA2* genes. HR-deficient cancers are sensitive to inhibitors of Poly-ADP Ribose Polymerase (PARP), and PARP inhibitors (PARPi's) have shown promising efficacy in the treatment of HGSOC. However, the majority of HGSOC patients will eventually develop resistance to PARPi's, and no overall survival benefit has been reported. Clinically, best characterized mechanism of PARPi resistance is restoration of BRCA1/2 protein expression accounting for 20-50% of clinical cases. Recently, emerging preclinical evidence has emphasized the role of replication fork protection in PARPi resistance. In addition, transcriptional regulation of the DNA damage response (DDR) proteins, including 53BP1, have been shown to contribute to PARPi resistance. However, the lack of understanding on the functional heterogeneity of the different PARP inhibitor resistance mechanisms has hampered the clinical targeting and development of biomarkers for acquired PARPi resistance in HGSOC.

RESULTS: To understand the dynamics and origin of PARPi resistance, we generated a *TP53-/-* and *BRCA1-/-* deficient epithelial (RPE) cell line using the CRISPR/Cas9 system. These cells are deficient in HR, and therefore are hypersensitive to PARPi *in vitro*. Using cyclic exposure of increasing concentrations of the PARPi Niraparib, we selected a cell population that were resistant to high concentrations (μM range) of the drug. We next isolated single cell clones from the resistant pool and performed deep functional profiling. As expected, the clones were resistant to multiple PARPi, including Niraparib, Olaparib and Talazoparib, and showed decreased DNA damage after PARPi treatment. However, none of the clones has restored BRCA1 protein expression. In functional assays, some clones had different levels of restored HR, and others showed a predominantly fork-stable, non HR-restored phenotype. Upon cytogenetic analyses, the parental RPE$^{TP53-/-BRCA1-/-}$ cells exhibited high clonal heterogeneity in terms of ploidy, whereas the PARPi resistant clones are mostly triploid. Interestingly, the HR-restored clones have lower levels of baseline chromosomal aberrations; however, mitomycin C induced chromosomal breaks in all the clones. In flow cytometry-based cell cycle profiling, the clones retained the G2/M accumulation upon PARPi treatment, similar to the parental RPE$^{TP53-/-BRCA1-/-}$ cells. Interestingly, all PARPi resistant clones show decreased levels of the DDR protein KAP1, and divergent expression levels of other DDR proteins, such as 53BP1. Importantly, the clones show significant heterogeneity in terms of sensitivity to cisplatin, as well as to DDR targeting agents, such as inhibitors of ATR and CHK1.

CONCLUSIONS: We have engineered *BRCA1-* deficient cells that are resistant to PARPi, and show that subclones of from these cells have significant functional heterogeneity in DNA repair dynamics, and are differentially vulnerable to DDR targeting agents. Importantly, our model system suggests that acquired PARP inhibitor resistance involves the adoption of several distinct resistance mechanisms that are potentially linked to DDR regulation. To enable clinical translation of our findings, we are in the process of performing genomic and transcriptomic profiling to discover the genomic evolution, common vulnerabilities, and novel biomarkers for acquired PARPi resistance in HGSOC.

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THE N6-METHYLATION OF ADENOSINE (M6A) IN FZD10 MRNA CONTRIBUTES TO RESISTANCE TO PARP INHIBITOR

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Chemical modifications of RNAs have emerged as a new layer of epigenetic gene regulation. N6-methyladenosine (m6A) is the most abundant chemical modification of messenger RNA. The m6A modification affects RNA fate and functions such as RNA stability. Despite the high initial clinical response rates to PARP inhibitors in BRCA1/2-mutated epithelial ovarian cancers, PARP inhibitor (PARPi) resistance remains a major challenge. The role of m6A modification in PARPi resistance has not previously been explored. Here we show that m6A modification of FZD10 mRNA contributes to PARPi resistance by upregulating the Wnt/β-catenin pathway in BRCA-mutated EOC cells. Global m6A profile reveals a significant increase in m6A modification in FZD10 mRNA. This correlates with an increase in FZD10 mRNA stability and an upregulation of the Wnt/β-catenin pathway in PARPi resistant cells. FZD10 knockdown or inhibition of the Wnt/β-catenin sensitizes the resistant cells to PAPRi. Mechanistically, downregulation of m6A demethylases FTO and ALKBH5 is sufficient to increase FZD10 mRNA m6A modification and reduce PARPi sensitivity. Moreover, PAPRi and Wnt/β-catenin inhibitor showed synergistic suppression of growth of PAPRi resistant cancer both in vitro and in vivo in a xenograft ovarian cancer mouse model. Our results show that m6A contributes to PAPRi resistance in BRCA-deficient EOC cells by upregulating the Wnt/β-catenin pathway through stabilizing FZD10. They also suggest that inhibition of Wnt/β-catenin pathway represents a potential strategy to overcome PARPi resistance.

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TARGETED INHIBITION OF HSP90 IMPAIRS DNA-DAMAGE RESPONSE PROTEINS AND INCREASES THE SENSITIVITY OF OVARIAN CARCINOMA CELLS TO PARP INHIBITORS

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Poly (ADP-ribose) Polymerase inhibitors (PARPi) are a promising class of inhibitors for the treatment of high grade serous ovarian carcinoma (HGSOC). The greatest activity of these agents is seen in patients with defects in DNA damage repair (DDR) mechanisms, including mutation or epigenetic inactivation of *BRCA1* and *BRCA2* genes and alterations in expression and/or function of DNA repair genes/proteins. PARPi are approved as second-line and maintenance therapies in recurrent HGSOCs. Notably, clinical trials have shown that single agent PARPi show activity in a significant number of HGSOC patients in the absence of alterations in *BRCA* genes, particularly in patients with platinum sensitive disease, possibly those with tumors exhibiting defects in homologous recombination (HR), or 'BRCAness'. To extend the benefit of these agents beyond patients with inherent defects in HR, we wished to test the idea that combination of PARPi with agents that functionally abrogate HR could extend the benefit of PARPi's. An attractive molecular target for this purpose is heat shock protein 90 (HSP90) based on its essential role in mediating the maturation and stability of several key proteins required for the DDR. The goal of this study was to test the hypothesis that targeted inhibition of HSP90 with a small-molecule inhibitor ganetespib (STA-9090) would sensitize non-*BRCA* mutant OC cells to the PARPi talazoparib (BMN-673). To test this hypothesis, we used established OC cell lines (OVCAR3, UWB1.289), and novel OC cells lines (OC-38, OC-1) derived in our laboratory from de-identified tumors isolated from patients with HGSOC. Cells were treated with talazoparib and ganetespib alone and in combination, and the effects of drug treatment on expression of DDR proteins, ionizing radiation (IR)-induced $RAD51^+$ and $\gamma H2AX^+$ foci, and cell viability was assessed. Ganetespib treatment led to dose- and time-dependent depletion of HSP90 client proteins participating in DDR including BRCA1 and 2, MRE11, CDK1, CHK1, RAD51. Treatment with ganetespib also led to a significant reduction in the percentage of $RAD51^+$ nuclei following IR. The quantity of DNA double-strand break marker γH2Ax foci/nucleus decreased over time in vehicle treated, but not in ganetespib-treated cells. We next conducted a comprehensive analysis of cytotoxicity in cells treated with ganetespib and talazoparib alone, in combination and at differing molar ratios. Combination indexes (CI) were calculated to assess additive, synergistic and antagonistic effects using the methods of Greco plus the bootstrap to determine statistical significance. Ganetespib sensitized *BRCA1*-null UWB1.289 cells to the effects of talazoparib (CI=0.73, $p<0.0005$). Among the non-*BRCA* mutant cell lines analyzed, the combination of ganetespib and talazoparib were synergistic in some patient-derived cell lines, but antagonistic in others. Together, our data suggest that ganetespib effectively disrupts critical DDR pathway proteins in HGSOC cells and may sensitize non-*BRCA*-mutant OC cells to PARPi. From clinical perspective, this implicates the potential of sensitization of some HGSOC patients without HR pathway alterations to PARPi, and potentially other DNA-damage inducing agents.

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ERADICATION OF CHEMORESISTANT METASTATIC OVARIAN CANCER WITH IMMUNOTARGETED EPIGENOMIC NANOSURGERY (IEN)

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INTRODUCTION: Resistance to conventional chemotherapy such as cisplatin, and radiotherapy in ovarian cancer may be caused by deregulated oncogenic miRNA-221/222 inhibiting induction of apoptosis.

METHODS: Chemoradioresistant cells overexpressing EphA2 were obtained for developing an ovarian metastatic cancer model, which was treated with anti-EphA2 MAbs immunotargeted pegylated nanosomes with encapsulated paired guide-RNAs targeting 3' and 5 ' of the precursor microRNA 221/222.

RESULTS: Post-treatment, the paired guide-RNAs caused deletions in the targeted pre-microRNA 221/222 genomic-sequences removing the stem-loop hairpins after blockade of RNase-III Drosha and DGCR8(microprocessor-complex) , subsequent transport by exportin-5/RanGTP and cleavage by Dicer inhibiting miRNA221/222 biogenesis, and miRNP circumventing their incorporation in RISC, and subsequent mRNA cleavage of coding genes regulating according to NGS the PTEN, GJA1, SEMA3B, TIMP3, PTPRM, CDKN1C,MGMT, CDKN1B, PUMA, connexin-43 (CX-43), c-Jun, MMP, NF-kB, PI3K, p27/Kip1, PTPμ, NIAP, ICAM-1, STAT1, STAT2, STAT3/Akt, CDK4, CDKN1C/p57, p53, and p57 inhibiting metastatic ovarian cancer cell proliferation, chemo/radioresistance, migration, angiogenesis, invasion, metastasis and maintenance of MET expressing ovarian metastatic cancer stem cells , while inducing cytotoxic immune responses (CTLs), and D2 irreversible stage of apoptosis or programmed cell death type-I leading to a bystander killing effect (BKE) due to phagocytosis of adjacent tumor cells according to transmission electron micrographs.

CONCLUSION: Immunotargeted epigenomic nanosurgery may eradicate metastatic ovarian cancer after inhibition of oncogenic miRNA 221/222 expression, and downstream signaling pathways involving interactions with coding RNA genes leading to apoptosis or PCD type I and BKE after the circumvention of chemo/radioresistant mechanisms.

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FABCLAVINE, A SECONDARY METABOLITE FROM XENORHABDUS BUDAPESTENSIS AS THERAPY AGAINST OVARIAN CANCER

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Ovarian cancer is the fifth most common cause of cancer-related deaths in women in the United States. Most women with ovarian cancer present with advanced disease. Even though initial response to surgery and chemotherapy is generally positive, many patients relapse with chemoresistant disease. We are especially interested in finding natural products that have anti-neoplastic activity against relapsed disease. Here, we report fabclavine, a secondary metabolite from Xenorhabdus budapestensis as novel anti-cancer agent.

Fabclavine was isolated and purified from cell-free supernatants of Xbu cultures. In a two-step procedure, active compounds were first precipitated from the supernatant with acetone and subsequently purified via reverse phase chromatography. Proliferation assays conducted on several ovarian cancer cell lines such as OVCAR3, OVCA433, ID8, IOSE, show that fabclavine inhibits the cancer cell proliferation at nanomolar (250-400nM) concentration but has no effect on IOSE at that concentration. The ovarian cancer cell line OVCA33 when tested in proliferation assay showed, they are sensitive to fabclavine at a ten-fold lower concentration than cisplatin. Besides proliferation assay in 2D culture, we also tested the effect of fabclavine in 3D culture of ovarian cancer cell lines. The results indicate, fabclavine was equally effective in 3D culture and killed 40-50% of the cells in spheroids. In addition, fabclavine was also tested against cancer stem cells isolated from ovarian cancer patient and grown in 3D culture and it inhibited the growth of stem cells by 45%. Treatment of ovarian cancer cell lines with Fabclavine indicated DNA damage and an increase in cleaved caspase 3 and Annexin V binding, confirming that this agent was killing the OVCAR3, ID8 and OVCA433 cancer cell lines via apoptosis. Further experiments are underway to determine the apoptosis pathway (intrinsic or extrinsic) activated by fabclavine. Here we are describing for the first time a novel agent which is water soluble, active at low concentration and conveniently purified from cell free supernatant of bacterial culture. These properties of fabclavine makes it an excellent molecule to develop as therapy against ovarian tumors.

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QUANTITATIVE HIGH-THROUGHPUT SCREENING USING AN ORGANOTYPIC MODEL IDENTIFIES TWO KINASE INHIBITORS THAT INHIBIT OVARIAN CANCER METASTASIS

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The cancer cells, stromal cells and stroma in the tumor microenvironment contribute to cancer metastasis and drug resistance. Therefore we applied an organotypic, robust and reliable assay for quantitative high throughput screening (qHTS) in drug discovery. This 1536-well HTS culture contains primary human mesothelial cells, fibroblasts, fibronectin and collagen type I and reproduces the human ovarian cancer (OvCa) metastatic microenvironment. The qHTS assay identifies small molecule compounds that inhibit OvCa adhesion/migration/invasion to the organotypic metastatic microenvironment.

Over 44,000 small molecules were screened in the primary qHTS assay. A counter screen was performed to eliminate any compounds toxic to the mesothelial cells or fibroblasts within the metastatic microenvironment. The activity of hit compounds was confirmed using five OvCa cell lines, and validated in secondary in vitro and in vivo biological assays. The active compounds directly inhibit at least two of three OvCa functions: adhesion, invasion and growth. In vivo, these compounds prevent OvCa adhesion/invasion and metastasis, and improve survival in mouse models.

Collectively, these data indicate that the three compounds, two tyrosine kinase inhibitors and one novel compound, identified using an complex organtoypic culture of the tumor microenvironment for qHTS are potential therapeutics for OvCa metastasis.

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WINDOW OF OPPORTUNITY TRIAL: ASSESSING THE ADAPTIVE RESPONSE OF HGSOC TO PARPI FOR INFORMED COMBINATION THERAPIES

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At least 50% of high grade serous ovarian cancer (HGSOC) patients display defects in the components of the homologous recombination (HR) DNA repair pathway, which is the only high fidelity DNA repair mechanism for double strand breaks (DSB). This provides an exciting opportunity for targeted therapies using drugs such as poly (ADP-ribose) polymerase inhibitors (PARPi). PARP is an important component of the base excision repair and alternative non-homologous recombination DNA repair pathways. Therefore, inhibition of PARP enzymatic activity induces DNA damage and leads to synthetic lethality in HR defective tumors. Several clinical trials with PARPi have shown an improvement of progression-free survival in patients but have only shown a limited effect on overall survival. As for many other targeted therapies, the cancer cells rapidly adapt to the stress induced by the drug and develop resistance. Resistance can either be genomic or adaptive. In adaptive resistance the cellular networks are rewired in the absence of genomic changes to mediate resistance. To overcome this resistance, several groups, including ours, suggested the use of PARPi-based combination therapies. Several clinical trials are currently testing PARPi combinations with drugs targeting the immune checkpoint, the DNA damage checkpoint, the PI3K pathway, and the MAPK pathway. Although several of these combinations are promising, the main challenge remains in finding the right drug combination for the right patient since each tumor has the potential to adapt differently to PARPi. We thus implemented a window of opportunity trial to determine whether it is possible to identify different adaptive responses to PARPi by comparing pre and post-treatment tumor samples from multiple sites in the peritoneal cavity. We used reversed phase protein array (RPPA) analysis to measure the expression of over 300 proteins in each tumor samples and compared pathways activity in pre and post treatment samples from individual patients. We also compared the adaptive response detected in cancer patients with the one observed in cell lines. Base on pathway scores, we developed an approach to predict which combination with PARPi would be most likely to benefit a specific patient. Overall, our results indicate that the adaptive response to PARPi treatment can be detected early during treatment and that individual patients utilize different pathways to adapt to the stress induced by the drug. This study reinforces the need and opportunity for informed combination therapies to improve outcomes in this devastating disease.

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NOVEL ANTIBODY DRUG CONJUGATE (ADC) AND COMPANION DIAGNOSTICS AGAINST CD248+ CANCER

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Antibodies recognizing specific tumor targets can be used to deliver therapeutic payloads with high degree of specificity. The simultaneous use of antibody against same target for imaging and for therapy, an approach named “theranostics”, offers unique opportunities for precision medicine and rapidly assessing therapeutic response at molecular level. The first example of such antibody-based theranotics is Herceptin in HER2-positive breast cancer: Herceptin is used as companion diagnostic imaging tool as well as the targeted therapy.

To develop effective anti-tumor therapeutics, one needs a robust biomarker, AND a good antibody that can specifically bind to the target. We and others have found one for EOC: CD248/TEM1. It is present at high level in tumor and its microenvironment in EOC and almost absent in normal organs. Importantly, CD248 is expressed by >90% EOC patients on the tumor or tumor vasculature, and its high expression correlates with poor survival. This means that if we can target it, we may destroy tumor but leave normal tissues intact. We also have developed robust antibody against CD248 (78Fc panel, US Patent 61/639,325). We showed that our patented antibodies can find the biomarker and upon binding deliver a payload, for PET imaging, near-infrared optical imaging or therapeutic, to CD248-expressing cells. Different toxin payload, such as auristatin and MD117, were conjugated to the antibody, and such ADCs were tested in vitro and in vivo in mice models. Surprisingly, we found such immunotherapies only directly kill, but also induce significant lymphocyte infiltration into tumors. In addition, tumor rejection was observed upon re-challenging in preclinical models. Such immune activation we observed could in turn sensitizing previously “cold tumor” EOC, to checkpoint inhibitors such as PD-1/CDLA4.

The long term goal is to develop companion diagnostics and antibody-immunotherapy: screening methods (such as antibody-based immunoPET and serum ELISA) to identify patients with EOC that can be treated with anti-CD248 drugs; and develop drugs that are linked to the antibody (such as antibody-drug conjugate) so it can be delivered to the tumor directly to kill. In addition, combined with PD-1 blockade, ADC therapies may exert antitumor immune response to sensitize “cold” tumors that previously resistant to PD-1 therapy.

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PARPI-INDUCED ALDH1A1 EXPRESSION CONTRIBUTES TO PARPI RESISTANCE IN OVARIAN CANCER CELLS

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Ovarian cancer (OC) is one of the most lethal female malignancy which accounts for just 2.5% of female cancer cases but 5% of deaths because of low survival. 90% of OC are epithelial ovarian cancer (EOC), with an overall 5-year relative survival rate of 47% and only 29% for patients diagnosed with distant-stage. 20% of OC cases are estimated to be due to inherited mutations that confer elevated risk, especially cancer susceptibility genes BRCA1 and BRCA2. PARP inhibitors (PARPi) are novel and promising cancer-targeted drugs. PAPRi are approved by FDA for clinical treatment of advanced EOC patients with BRCA1/2 gene mutation. However, patients gradually gained resistance to PARPi with continuously increased recurrence rate (>90%). Thus, understanding the mechanism underlying PARPi resistance is an urgent need for improving the PARPi efficacy. Aldehyde dehydrogenase (ALDH) activity is considered as a cancer stem cell (CSC) marker and also relative to drug resistance. However, the relationship between ALDH activity and PAPPi resistance remains unclear. In this study, we generated two olaparib-resistant EOC cell lines by continuously treating BRCA2-mutated PEO1 and Kuramochi cell lines for 6 months, and found that these resistant cell lines exhibited higher ALDH activity compared to their corresponding parent cell lines. In addition, short-term treatment of PEO1 and Kuramochi cells with olaparib (7 days) also increased the ALDH+ cell population in these cells, and olaparib-induced ALDH--to-ALDH+ conversion contributed to the expansion of the ALDH+ cell population after olaparib treatment. qRT-PCR analysis demonstrated that ALDH1A1 is the major gene in the ALDH gene family that was induced by olaparib. Overexpression of ALDH1A1 increased olaparib resistance in a panel of EOC cells lines including both BRCA2-muated and BRCA2-wild type cell lines. In summary, our data indicate that olaparib is able to induce ALDH1A1 gene expression, which results in the enhanced ALDH activity. The enhanced ALDH activity can contribute to olaparib resistance in BRCA2-mutated EOC cells.

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QUADRA: A PHASE 2, OPEN-LABEL, SINGLE-ARM STUDY TO EVALUATE SINGLE-AGENT NIRAPARIB TREATMENT IN PATIENTS WITH RELAPSED OVARIAN CANCER (ROC) WHO HAVE RECEIVED ≥3 PRIOR CHEMOTHERAPY REGIMENS

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BACKGROUND: Patients with ROC have limited treatment options in later lines of therapy, as many become platinum-resistant or -ineligible. Poly(ADP-ribose) polymerase inhibitor (PARPi) treatment is one option, however these agents are currently not approved for *BRCA*wt patients in the treatment setting. Niraparib, a PARPi, has demonstrated increased progression-free survival vs placebo as maintenance therapy for platinum-responsive ROC. Niraparib was effective regardless of *BRCA*mut or homologous recombination deficiency (HRD) status, although an increased treatment effect was observed in the HRDpos population. QUADRA (NCT02354586) evaluated niraparib treatment in heavily pretreated ROC patients, regardless of *BRCA*mut or HRD status, platinum sensitivity, or prior PARPi use.

METHODS: Eligible patients had grade 2-3 serous ROC, ≥3 prior chemotherapy lines, and measurable disease. Patients were evaluated for *BRCA*mut and HRD status (MyChoice HRD Test). Patients received niraparib 300 mg once daily until progression; dose could be lowered to 200 mg or 100 mg due to treatment-emergent adverse events (TEAEs). The primary endpoint was objective response rate (ORR) per RECIST v1.1 in patients treated in 4th or 5th line who were HRDpos, platinum sensitive, and PARPi naïve.

RESULTS: 463 patients were treated. Of these, 161 were platinum refractory, 151 platinum resistant, and 120 platinum sensitive to their last line of platinum therapy. Of note, 83 patients were primary platinum resistant or refractory (42 and 41, respectively). Median number of prior lines of therapy was 4 (range 2-16), and 27% of patients were treated in 6th or later line. 100% of patients received prior platinum, 99.6% received prior taxane, 81% received prior doxorubicin, 63% received prior bevacizumab, and 59% received prior gemcitabine.

In the primary efficacy population (N=45), ORR was 29% (95% CI: 16-44, ***P***=0.0003), disease control rate (DCR) was 71% (95% CI: 56-84), and duration of response (DOR) was 9.2 months (95% CI: 5.9-NE); overall survival (OS) was not reached (95% CI: 18.5-NE). The efficacy outcomes in all 456 patients with measurable disease at baseline treated in 4th or later line demonstrated a DCR of 49% with 2 complete responses (CR), 36 partial responses (PR), and 187 patients with stable disease (SD). The responses were durable with a median DOR of 9.4 months, a clinical benefit rate (CR+PR+SD) at 16 weeks of 29%, and median OS of 17.2 months (95% CI: 14.9-19.8) among all patients treated in 4th or later line.

266 (57.5%) patients had grade ≥3 treatment-related TEAEs. Grade ≥3 thrombocytopenia event (thrombocytopenia or decreased platelet count) by dose at onset of the event was 27.5% at 300 mg, 4.7% at 200 mg, and 2.7% at 100 mg. The other most common grade ≥3 TEAEs were anemia (26.3%) and neutropenia (8.2%). Hematologic TEAEs were most frequent in the first month and decreased in frequency and severity after dose reduction during months 2-3.

CONCLUSIONS: Niraparib demonstrated durable anticancer activity in this heavily treated (≥3 lines) ROC population, including platinum-resistant or *BRCA*wt patients. Toxicities were consistent with previous niraparib studies.

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CHEMICALLY INDUCED HYPOXIA PROMOTES OVARIAN CANCER CHEMORESISTANCE

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The accumulation of cells, derived from the imbalance between the aberrant proliferation and resistance to apoptosis of cancer cells, leads to an increased consumption and consequently decreased availability of oxygen (hypoxia). Hypoxia Inducible Factor (HIF-1) is the key regulator of cell adaptive response under different extracellular stimuli. Subunit HIF-1α is kept at low levels under normal oxygen supply, but strongly upregulated under hypoxia. HIF-1α is overexpressed in several malignant and metastatic tumors but its significance in ovarian cancer prognosis is still controversial. $CoCl_2$ is a chemical compound widely used as a positive control for the upregulation of HIF-1α by preventing its proteosomal degradation. Acquired chemoresistance is considered one of the main factors associated with poor prognosis and survival in ovarian cancer patients, and therefore, its understanding is nowadays one of the major challenges pursued to facilitate treatment and increase life expectation of patients. The aim of this study was to determine the possible role of HIF-1α in the development of chemoresistance in ovarian carcinoma. A27801A9 ovarian cancer cell line was treated with 100 μM $CoCl_2$ for 3, 6 12 and 22 hours for chemical induction of hypoxia. Response to cisplatin and paclitaxel chemotherapeutic drugs was tested and compared with untreated cells (normoxia). Western blot was used to test epithelial-to-mesenchymal transition (EMT) and lysyl oxidase (LOx) markers, and enzyme-linked immunosorbent assay (ELISA) to test the secretion of cytokines in cell supernatant. Chemical hypoxia resulted in paclitaxel resistance in a dose-dependent manner, with 7 to 19% increased viability for hypoxic cells in relation to normoxic cells. No effect was observed for cisplatin. HIF-1α expression achieved the maximum level at 12 hours. Mesenchymal markers N-cadherin and vimentin, as well as the transcription factor snail were not remarkably affected, while epithelial marker E-Cadherin expression was reduced at 22 hours and transcription factor slug presented the maximum expression at 12 hours, as well as lysyl oxidase. Tumor necrosis factor (TNF-α) was not affected by the treatments, while inflammatory interlukins IL-6, IL-10 and IL-1β showed differentiated responses, with a slight increase of 3 pg/mL of IL-10 at 12 hours, a decrease of IL-6 in a time dependent manner (from 40 to 3 pg/mL), and a decreased secretion of IL-1β (around 50%) in relation to the control for all the treatment times. These results suggest that epithelial morphology may begin to be lost at 12 hours, through downregulation of E-cadherin mediated by slug as a consequence of HIF-1α upregulation. In addition, the differential expression of cytokines suggests that HIF-1α may be involved in the cellular immune adaptive response. The enhanced mitotic capacity that has been associated to LOx, was possibly increased due to HIF-1α upregulation, reducing the effectivity of paclitaxel and contributing to a consequent acquired chemoresistance.

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TARGETING OVARIAN TUMORS WITH OXPHOS INHIBITORS

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Aerobic glycolysis is an important metabolic adaptation in tumors. However, there is mounting evidence that oxidative phosphorylation (OXPHOS) also contributes to metabolism in cancer cells. Therefore, agents targeting OXPHOS can serve as novel anticancer agents. We will present data on small molecule agents that inhibit the proliferation of ovarian and other cancer cells by inhibiting mitochondrial electron transport. We have focused our attention on citral, plumbagin, curcumin, cinnamaldehyde, piperlongumine, perillaldehyde, nonenal and other naturally occurring agents. All of these molecules share a common molecular epitope, the unsaturated carbonyl that participates in redox reactions. Cancer cells treated with these agents show a marked decrease in oxygen consumption rate and an increase in extracellular acidification. The redox ratio (NADH/FAD) and cellular ATP levels also decrease in response to these small molecule agents. In our search for additional and more potent OXPHOS inhibitors we have discovered that the FDA-approved anti-malarial drug atovaquone is an efficient inhibitor of electron transport in ovarian cancer cells. Chronic administration of atovaquone decreases the growth of ID8 tumors in C57BL/6 mice. Investigation in to the mechanism of action of the unsaturated carbonyl compounds indicates that their initial effect in cancer cells is the immediate and substantial rise in oxygen radicals as a result of OXPHOS inhibition. This increase in reactive oxygen causes extensive oxidative damage leading to DNA strand breaks. Subsequently, we have observed activation of p53-mediated apoptosis. These experiments are suggesting that inhibition of OXPHOS and the subsequent increase in intracellular oxygen radicals can result in the reactivation of the tumor suppressive responses of at least a subset of the p53 mutants. Additionally, our studies also indicate that the oxidative stress occurring as a result of OXPHOS inhibition directly leads to inhibition of ion transport through the Na+/K+-ATPase. As a result of this inhibition, cancer cells are unable to maintain a normal membrane potential. Studies are currently underway to determine if the inability to maintain membrane potential also contributes to apoptotic cancer cell death induced by these unsaturated carbonyl-containing compounds. Oxidative stress caused by these compounds leads to an increase in superoxide dismutase, catalase and glutathione synthesis via the activation of Nrf-2. Inhibition of Nrf-2 results in an increase in the potency of the unsaturated carbonyl compounds. We are therefore examining Nrf-2 increase as a form of chemoresistance mechanism that likely impinges on the anti-cancer activity of the unsaturated carbonyl compounds. These studies are allowing us to develop medicinal chemistry-based approaches to develop novel molecules with increased potency and targetability as OXPHOS inhibitors for the treatment of ovarian cancer.

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SYNERGISTIC COMBINATION OF MORTALIN-TARGETING AND P53 REACTIVATOR DRUGS FOR OVARIAN CANCER

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PURPOSE: TP53 gene missense mutations represent the most common genetic defect in fallopian tube (FT) lesions and high grade serous ovarian cancer (HGSOC) and cause of drug resistance. We hypothesized that drugs with complementary p53 protein-targeted mechanisms (SHetA2 drug release of p53 from mortalin binding, PRIMA-1^{MET} drug reactivation of p53 transcription activity) synergistically kill ovarian cancer cells without harming normal cells.

EXPERIMENTAL DESIGN: SHetA2-mediated cytoplasmic to nuclear/mitochondrial p53 translocation was studied in wildtype and mutant p53 ovarian cancer cell lines using Western blot analysis of subcellular-enriched fractions and fluorescent microscopic imaging of immunocytochemistry. Human FT epithelial cells (hFTSECs) were used as healthy controls. The p53 reactivator drugs PRIMA-1 and PRIMA-1^{MET} were combined with SHetA2 to study drug interactions and mechanisms using Western blot, rtPCR, cytotoxicity, reactive oxygen species (ROS) and ATP assays.

RESULTS: SHetA2 induced p53 nuclear and mitochondrial accumulation in ovarian cancer cell lines, but not in hFTSECs. Both mortalin and p53 expression altered SHetA2 cytotoxicity in ovarian cancer cells. Modest effects of PRIMA-1^{MET} on cell lines inversely correlated with SLC7A11 expression, a known biomarker of PRIMA-1^{MET} sensitivity. SHetA2 induced SLC7A11 in the presence of wildtype or missense mutant p53, but inhibited SLC7A11 in the absence of p53. SHetA2 and PRIMA-1^{MET} were synergistic-to-strongly synergistic in ovarian cancer cells with mutant p53, synergistic with wildtype p53, additive with null p53 and antagonistic in hFTSECs. Elevated apoptosis, p53 transcription activity and ROS in combination with reduced ATP were associated with this mutant p53-dependent synergy.

CONCLUSION: Altogether, our findings identified the SHetA2 and PRIMA-1^{MET} combination as a promising new therapeutic strategy for the treatment of HGSOCs with missense mutant p53.

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ANTI-TUMOUR EFFECTS OF ALL-TRANS RETINOID ACID, AN ANNEXIN A2-S100A10 PATHWAY INHIBITOR ON SEROUS OVARIAN CANCER

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Annexin A2 is increased in serous ovarian cancer and plays an essential role in ovarian cancer invasion and metastasis. In combination with S100A10, annexin A2 plays an important role in the plasminogen activator system regulating plasmin production. The aim of this study was to investigate the potential utility of all-trans retinoid acid (ATRA), an inhibitor of the annexin A2-S100A10 signalling pathway, as a new therapeutic against serous ovarian cancer. Survival of serous ovarian cancer cells (OVCAR-3, OV-90, & OAW28) was significantly decreased by ATRA treatment (1-5μM). ATRA (1μM) also significantly decreased proliferation (Ki67 positivity, p=0.0034), S100A10 protein levels (p=0.0273), and increased cell apoptosis (cleaved caspase-3 positivity, p=0.0024) in serous ovarian cancer tissues using an ex vivo explant assay. In OAW28 cells, reduced cell survival following ATRA treatment was associated with a reduction of S100A10 protein levels, S100A10 and annexin A2 membrane localization, plasmin generation, motility and invasion. In contrast, ATRA inhibited OV-90 cell survival and invasion but did not affect plasmin activation or S100A10 and annexin A2 membrane localization or protein levels. These findings suggest that ATRA inhibits serous ovarian cancer proliferation and invasion via both S100A10 dependant and S100A10 independent mechanisms. Our results show that ATRA has promising potential as a novel therapy against serous ovarian cancer that warrants further evaluation.

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PIN1: A PROMISING TARGET FOR PLATINUM-RESISTANT HIGH GRADE SEROUS OVARIAN CANCER

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Epithelial ovarian cancer is the leading cause of death in gynaecological cancers in developed countries and the fifth most common cause of cancer mortality in women. High-grade serous epithelial ovarian cancer (HGS-EOC) is derived from the surface of the ovary and/or the distal fallopian tube: the relative contribution to the EOC remains to be determined. Currently, debulking surgery and chemotherapy are the standard therapy in ovarian cancer treatment. Evidence from different clinical trials established the paclitaxel and carboplatin combination regimen as the first-line chemotherapeutic treatment, although resistance occurs.

Pin1, a peptidyl prolyl cis-trans isomerase, which controls different oncogenes and tumor suppressor genes, is overexpressed in about fifty percent of HGS-EOC suggesting that it may be a potential therapeutic target. Although many efforts have been dedicated to the development of Pin1 inhibitors, up to now a few are effective on cancer cells or ***in vivo*** mouse models. Our group recently published the first liposomal formulation of a potent and specific Pin1 inhibitor, which successfully constrains tumor growth in a mouse model of ovarian cancer. We demonstrated the involvement of Pin1 in HGS-EOC showing that it is overexpressed in ovarian cancer tissue samples and when genetically or chemically inhibited, promotes ovarian cancer cell death ***in vitro*** and ***in vivo (PMID: 29746956)***. Spheroids derived from SKOV3 cell line were utilized to better reproduce the spreading of cancer cells from ascitic fluids to secondary sites. After Pin1 chemical inhibition, the cells fail to form spheroids or spheroid growth and migration. Since platinum-resistance occurs in almost all HGS-EOC patients, we investigated the role of Pin1 as effective advanced therapy in 2D and 3D ***in vitro*** systems. In literature, it is reported that the genetic inhibition of Pin1 synergizes with cisplatin in cervical cancer. Our preliminary data showed that chemical or shRNAs inhibition of Pin1 in HGS- EOC cell lines sensitize the cells to carboplatin. To investigate which molecular pathways are involved, different key cancer proteins were examined. Pin1 affects PI3K/Akt pathway, by decreasing the expression of activated Akt protein, a key regulator of cancer cell proliferation, migration and survival. In conclusion, we propose Pin1 as promising targeted therapy of HGS-EOC patients with platinum-resistance.

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CHEMORESISTANT OVARIAN CANCER STEM CELLS REVEAL NOVEL THERAPEUTIC TARGETS

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More than 60% of patients with ovarian cancer respond to first-line therapy, often achieving complete remission. However, these encouraging clinical outcomes are quickly overshadowed by high rates of treatment failure. More than half of advanced-stage patients relapse within five years, and these patients face poor prognoses. We sought to better understand the cause of ovarian cancer treatment failure with the hope of improving patient outcomes. We hypothesized that ovarian cancer stem cells may resist chemotherapy allowing them to regrow tumors causing relapse, metastasis and ultimately death. We further postulated that characterization of these cells would reveal therapeutic targets that may be exploited to achieve more durable remissions. To begin to test this hypothesis, we proposed that high aldehyde dehydrogenase I activity ($ALDH1^{high}$) would identify stem-like ovarian cancer cells that would resist chemotherapy treatment and be capable of regenerating tumors.

We compared $ALDH1^{high}$ cells to $ALDH1^{low}$ cells in three ovarian cancer cell lines: the human SKOV3 cell line, the rat FNAR-C1 cell line, and the murine ID8 cell line. Consistent with our predictions, we found that $ALDH1^{high}$ cells were resistant to several drugs commonly used to treat ovarian cancer. The $ALDH1^{high}$ population was also the only fraction of cells capable of forming tumors ***in vivo***. In keeping with their proposed cancer stem cell phenotype, $ALDH1^{high}$ cells were smaller in size, relatively quiescent, and able to regenerate the phenotypic diversity of the cell line. Furthermore, they lacked contact inhibition and were capable of nonadherent growth. Significant controversy exists concerning how best to identify ovarian cancer stem cells. Many markers have been proposed with data to both support and refute their use in the literature. Resolving these uncertainties is hampered by the lack of systematic evaluation of each marker in proposed cancer stem cell pools. We used microarray analysis and quantitative real-time RT-PCR to measure the expression of ABCG2, CD24, CD44, CD133 and KIT in our $ALDH1^{high}$ cells compared to their $ALDH1^{low}$ counterparts and found no consistent differential regulation in the $ALDH1^{high}$ cells across all three cell lines. Further work remains needed to more clearly define the relationships between each of these proposed ovarian cancer stem cell markers.

Gene expression profiles identified several possible vulnerabilities that could be exploited therapeutically. $ALDH1^{high}$ cells have upregulation of the mTOR pathway, FGF18 and CD47. They also express Her-2/neu at similarly high levels as $ALDH1^{low}$ cells. Drugs are already clinically available to target mTOR and Her-2/neu, which would speed their implementation. FGF18 signals through the FGFR3 receptor. Although FGF18 is upregulated in the cancer stem cell pool, both cancer stem and non-stem cells express its receptor, FGFR3, at similar levels. Several approaches to target FGFR3 are in clinical trials, as are antibodies targeting CD47. mTOR and CD47 inhibition would potentially be selective for ovarian cancer stem cells, but Her-2/neu and FGFR3 inhibition could also target the more differentiated, non-stem cancer cells. Further work is necessary to validate these treatment approaches.

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TARGETING BROMODOMAIN PROTEINS AS A THERAPEUTIC STRATEGY FOR CLEAR CELL CARCINOMA OF THE OVARY

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Background and Objectives: Clear cell carcinoma of the ovary (OCCC) is more resistant to platinum-based chemotherapy than high-grade serous ovarian cancer (HGSOC). Also, the indication for PARP inhibitors seems to be limited in OCCC because of the lower frequency of homologous recombination deficiency. As genomic background significantly differs among subtypes, therapeutic strategies should be optimized in response to each subtype or unique gene alterations. This study was designed to find novel therapeutic targets for OCCC by utilizing siRNA screens and unique patient-derived ovarian cancer cells that phenocopy original tumors.

Materials and Methods: Novel patient-derived ovarian cancer cells were established and kindly provided by Dr. Tan Ince at University of Miami. Genomic alterations of these cells were determined by DNA sequencing. siRNA screens targeting 2,172 unique genes were performed with ARID1A-mutated OCCC cell line OCI C5x, and high-grade serous ovarian cancer cell line OCI P5x. Genes inhibition of which significantly suppressed cell proliferation were selected as hits for each subtype independently. The difference of cell viability between OCI C5x and P5x was also compared, and subtype-specific hits were additionally determined. Hits were applied to Gene Ontology (GO) enrichment analysis to comprehend the gene set enrichment. Several prioritized targets for OCCC were validated with additional RNAi experiments and small molecule inhibitors.

Results: 114 and 108 genes were selected as hits specific to OCI C5x and OCI P5x, respectively. GO enrichment analysis revealed OCCC is potentially more vulnerable to epigenetic interventions than HOGSC. The following inspection revealed bromodomain-containing proteins were enriched in the hits for OCI C5x. Among these, we highlighted bromodomain and extra-terminal domain (BET) family proteins BRD2 and BRD3. Subsequent RNAi experiments validated BRD2 and BRD3 are highly potent therapeutic targets for OCCC.

Conclusions: siRNA screening results suggest different approaches are required according to subtypes for the targeted therapy of ovarian cancer. We propose that epigenetic intervention such as BET protein inhibition as a potential therapeutic option for OCCC, 50-60% of which harbor ARID1A mutation.

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VAL-083 (DIANHYDROGALACTITOL) SYNERGIZES WITH PARP INHIBITORS IN BRCA-PROFICIENT AND BRCA-DEFICIENT OVARIAN CANCER MODELS

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BACKGROUND: Ovarian cancer is normally treated with platinum (Pt)-based chemotherapy, but patients frequently develop Pt-resistance. Dysfunctional p53 is implicated in Pt-resistance, comprising a therapeutic challenge in high grade serous ovarian carcinoma (HGSC), where p53 is universally mutated (96%). Attempts to overcome Pt-resistance in HGSC include agents blocking the DNA repair pathways, most notably the PARP inhibitors (PARPi), leading to the accumulation of DNA double strand breaks (DSBs) and cancer cell death. Sensitivity to PARPi is correlated with deficiencies in the homologous recombination (HR) DNA repair system, which is in charge of accurately repairing DSBs. BRCA1 and BRCA2 are key proteins in HR, and mutated BRCA1/2 (present in 20% of HGSC) are well-established biomarkers for PARPi sensitivity. PARPi treatment of Pt-sensitive gynecologic malignancies have improved progression free survival (PFS); however, improvements in overall survival have not been achieved and a 5-year survival rate of only 40% remains in ovarian cancer. Additionally, resistance to PARPi in the clinical setting is emerging as a significant medical challenge. VAL-083 is a first-in-class bifunctional DNA damaging agent with demonstrated clinical activity against a range of cancers, including ovarian. VAL-083 rapidly induces interstrand cross-links at guanine-N7 leading to DSBs, activation of the HR repair pathway and cancer cell death. Notably, VAL-083 induces cell death through two parallel pathways - one p53-independent and one p53-dependent. We have shown that VAL-083 is able to overcome cisplatin-resistance in a panel of ovarian cancer in vitro models, independent of p53 status. We have also shown that VAL-083 maintains activity independent of prominent DNA repair mechanisms such as O6-methylguanine DNA methyltransferase (MGMT), non-homologous end-joining (NHEJ) and mismatch repair (MMR) implicated in resistance to numerous chemotherapeutics, including cisplatin and PARPi. Cancer cells thus rely heavily on functional HR pathway for repair of VAL-083-induced DSBs. This suggests combination therapy with agents further inducing DSBs or blocking their repair, including PARPi. Taken together, these data propose VAL-083's potential for targeting Pt-resistant HGSC and for combination treatment with PARPi.

METHODS: In this study, we examined the cytotoxicity of VAL-083 in combination with PARPi (olaparib, niraparib, rucaparib, veliparib or talazoparib) against HR-proficient and HR-impaired ovarian cancer cells. VAL-083 cytotoxicity alone and in combination with PARPi was investigated using the 5-day MTT assay in HR-proficient (control siRNA) and HR-impaired (BRCA1 siRNA knockdown) A2780 ovarian cancer cells. RESULTS: We report increased VAL-083 cytotoxicity against HR-impaired A2780 cancer cells. We further report synergy between VAL-083 and PARPi olaparib, talazoparib and niraparib in both HR-proficient and HR-deficient settings. VAL-083 combination with rucaparib produced synergistic cytotoxicity in the HR-deficient setting, while VAL-083 combination with veliparib was no more than additive. These data demonstrate that VAL-083 can synergize with some PARP inhibitors in both a HR-proficient and HR-deficient setting.

CONCLUSION: Our results demonstrate a distinct DNA damaging mechanism for VAL-083, resulting in the ability to overcome cisplatin-resistance, target HR-impaired tumors and overcome MGMT, MMR and NHEJ-related chemoresistance. In addition, VAL-083 synergized with several PARPi, particularly olaparib, rucaparib and talazoparib, in both HR-proficient and HR-deficient ovarian tumor cells.

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EFFICACY OF NOVEL TOPOISOMERASE 1 INHIBITORS AND THEIR SYNERGY WITH PARP INHIBITORS IN A MURINE MODEL FOR OVARIAN CANCER

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Camptothecin derivatives topotecan and irinotecan are currently the only FDA-approved topoisomerase I (Top1) inhibitors. Despite their wide usage they have many drawbacks: chemical instability, short half-life, dose-limiting bone marrow and gastrointestinal toxicity, and they are drug efflux substrates. The novel non-camptothecin Top1 inhibitors, indenoisoquinolines (LMP400, LMP776 and LMP744), have been developed to overcome these limitations. We explored whether treatment with LMPs could induce "synthetic lethality" in cells with impaired homologous recombination (HR) repair mechanism and whether it could be enhanced by combining them with PARP inhibitor, olaparib. Using the DT40 cell line, we assessed in vitro the role of HR in the cellular responses to the LMPs. We found that BRCA1-, BRCA2- and PALB2-deficient cells are 3 to 5 times more sensitive to the LMP compounds than wild type cells. Moreover, combination treatments showed a significant synergy between each of the three LMPs and olaparib. To confirm the differential response of Brca1 wild type or Brca1 deficient cells to LMP treatment we assessed compound potency in primary ovarian cancer cell lines derived from a genetically engineered mouse (GEM) model for serous epithelial ovarian cancer (SEOC). As in DT40 cells, we observed that murine Brca1 deficient cells were more sensitive to all 3 LMP compounds than Brca1 wild type cells. Our results suggest that Brca1 deficiency renders cells more sensitive to treatment with LMPs or combination of LMPs and olaparib, potentially directing selection of patients with impaired HR repair mechanism in the phase 2 clinical trials that are in preparation. To confirm this observation in vivo, we are performing efficacy studies using LMP compounds alone or in combination with olaparib in orthotopic allograft mouse models for ovarian cancer derived from the GEM model. In Brca1 deficient tumors, treatment with each of the 3 LMP compounds led to significant increase of survival compared to vehicle treated group. LMP400 had the greatest beneficial impact on survival and tumor regression. To confirm synergy between LMPs and olaparib we selected LMP400 to represent the LMPs and repeated the efficacy study using Brca1 deficient tumor model. We observed that suboptimal doses of LMP400 or olaparib used as single treatments resulted in some increase in survival, however, when combined they significantly increased survival due to tumor regression. Our data demonstrate that the LMPs synergize with olaparib and provide a rationale for personalized treatment and Phase 2 clinical trials with the indenoisoquinolines in combination with PARP inhibitors in HR-deficient ovarian cancers.

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THE ANTIPROGESTIN/ANTIGLUCOCORTICOID MIFEPRISTONE AND THE HIV PROTEASE INHIBITOR NELFINAVIR CAUSE ENDOPLASMIC RETICULUM STRESS AND POTENTIATE THE TOXICITY OF PROTEASOME INHIBITION IN HIGH-GRADE SEROUS EPITHELIAL OVARIAN CANCER CELLS

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Epithelial ovarian cancer (EOC) is a fatal disease due to late diagnosis and lack of effective long-term treatment(s). Since the introduction of debulking surgery and platinum (Pt)-taxane (Tx) therapy over 30 yrs. ago, there has been no significant breakthrough impacting the overall survival of these patients. Though over 70% of diagnosed women respond to front-line standard of care with remission, the disease hides as microscopic (minimal residual) within the abdominal cavity for about 18-24 months (mo.), recurring thereafter with a phenotype usually not responsive to current chemotherapeutic agents. As patients are left without treatment between remission and recurrence, our research initiative is to develop a consolidation therapy for chronic use after standard of care. In this work we explored whether such consolidation therapy could be developed from two simultaneous strategies: proteasome inhibition and aggravation of the stress of the endoplasmic reticulum (ER). The rationale for this combination therapy is that malignant cells are more susceptible to the toxicity of proteasome inhibition and operate with increased expression of ER stress-related proteins—coined as unfolded protein response (UPR) addiction—allowing cancer cells to survive in a hostile environment of reduced nutrients, acidosis, energy deficiency, and low oxygen tension (hypoxia). We hypothesized that simultaneous ER stress aggravation and blockage of the proteolytic capacity of the proteasome should cause sufficient ER stress to tilt cells to a death fate. We report that antiprogestin/antiglucocorticoid mifepristone (MF) as well as HIV protease inhibitor nelfinavir (NFV) enhanced the stress of the ER in EOC cells of high-grade serous origin (HGSOC), which is the most aggressive subtype of EOC, represents the majority of cases of EOC, and causes 2/3 of all deaths from this disease. We demonstrated, in HGSOC cells, that both MF and NFV cause cell cycle arrest associated with increased expression of cyclin dependent kinase inhibitor p27kip1, while triggering the UPR in a dose-dependent manner assessed by increased subrogate ER stress biomarkers GRP78, ATF4, and CHOP. We also discovered that blocking the ubiquitin proteasome system (UPS) using cytostatic concentrations of the proteasome inhibitor bortezomib (BZ) causes accumulation of poly-ubiquitinated proteins and further activation of the UPR. More importantly, when we combined BZ with either MF or NFV, we observed a potentiation of the action of BZ leading to EOC lethality. These results suggest that targeting the ER stress-associated protein quality control machinery with either an antiprogestin/antiglucocorticoid agent or an HIV protease inhibitor may provide an alternative chronic treatment approach against HGSOC after standard of care. In addition, the data provide the rationale for rapid repurposing to treating HGSOC, of three drugs clinically approved for other uses, as their systemic toxicities have already been assessed.

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APTAMER CONJUGATED NANOPARTICLES FOR TARGETED DRUG DELIVERY

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Targeted Delivery of therapeutic drugs and imaging agents, using nanoparticles, shows great promise in diverse fields, including the diagnostics and treatment of cancer. Monoclonal antibodies (mAbs) that have been traditionally used for detecting specific proteins can also be used as targeting moieties. However, due to their intrinsic characteristics such as immunogenicity, batch-to-batch variation and instability, antibodies have several limitations for in vivo applications. Nucleic acid based aptamers are emerging as attractive alternatives for mAbs. We have developed single-stranded DNA aptamers, which can specifically recognize and bind to ovarian cancer endothelial cells. Using mass-spectroscopy based proteomics, we have identified Annexin A2 as the protein target for the selected aptamers. These aptamers can act as homing devices to deliver therapeutic drugs and/or imaging agents specifically to ovarian cancer cells. Using a highly stable, three-way junction (3WJ) motif from the phi29 packaging RNA as a scaffold, we made a multi-functional RNA nanoparticle that can harbor therapeutic drugs, imaging agent, siRNA and the targeting ligand. The aptamer-containing multi-functional RNA nanoparticle is used to deliver Doxorubicin to Annexin A2 positive ovarian cancer cells, while the control nanoparticles failed to deliver. After systemic injection in mice, the DNA/RNA hybrid nanoparticles remained intact and strongly bound to tumors with little accumulation in healthy organs 6 hour post-injection. The aptamer-3WJ-Sph1/Dox intercalates selectively enhanced toxicity to Annexin A2 positive ovarian cancer cells in vitro, demonstrating the potential of the constructed DNA/RNA hybrid nanoparticles to enhance the therapeutic efficiency of Doxorubicin at low doses for ovarian cancer treatment. The use of the aptamer-3WJ RNA nanoparticle will benefit ovarian cancer patients by reducing the side effects of cancer chemo-therapeutics and increasing its local concentration in the tumor microenvironment after systemic administration. Our ultimate goal is the development of patient-optimized aptamer-nanoparticle delivery systems for chemotherapy and molecular diagnostics imaging.

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SC-003, AN ANTIBODY-DRUG CONJUGATE TARGETING DIPEPTIDASE 3, EXHIBITS POTENT ANTI-TUMOR ACTIVITY IN PATIENT-DERIVED XENOGRAFT MODELS OF HIGH GRADE SEROUS OVARIAN CANCER

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Ovarian cancer describes a group of malignancies that remain a serious threat to women's health and claim more than 14,000 deaths in the US each year. Among these, high-grade serous ovarian cancers (HGSC) represent the most common and aggressive form, and tumor recurrence is near-universal following initial response to carboplatin and paclitaxel. While multiple treatment options are available for platinum-sensitive recurrent HGSC, such as continued use of carboplatin/paclitaxel with or without bevacizumab, as well as several FDA-approved PARP inhibitors, there are few effective treatment options for platinum-resistant HGSC. Its inherent heterogeneity, characterized by genomic instability and numerous DNA copy number aberrations, poses challenges to the discovery of novel therapeutic targets.

Patient-derived xenograft (PDX) models are an important platform for target identification and efficacy testing of therapeutic agents, as they sustain the intratumoral heterogeneity observed in advanced human tumors more faithfully than established cell lines. The isolation of distinct tumor cell populations by flow cytometry and subsequent testing of their tumorigenic potential can identify tumor-initiating cells (TICs), which have the potential to propagate the tumor over multiple generations in immune-deficient NOD/SCID mice. Thorough characterization of these TIC populations by transcriptome profiling and flow cytometry has identified TIC-enriched cell surface proteins that are targetable by antibody drug conjugates (ADCs), including EFNA4 in mixed Müllerian and ovarian cancers and PTK7 in triple-negative breast and ovarian cancers. ADCs consist of a monoclonal antibody directed against a cell surface epitope linked to a cytotoxic agent, such as auristatin, maytansinoid, or pyrrolobenzodiazepine (PBD).

Here, we describe the generation and characterization of a PDX bank for ovarian cancers that led to the identification of dipeptidase 3 (DPEP3) as a TIC-associated target in HGSC. We show that DPEP3 is enriched in the TIC fraction of platinum-sensitive and platinum-resistant HGSC PDX models, where it localizes to the plasma membrane and is detected by flow cytometry and immunohistochemistry (IHC). In contrast, DPEP3 expression is low or absent in most normal adult tissues, thus providing a therapeutic window for an antibody-based therapeutic agent. In order to target DPEP3-expressing ovarian cancer cells, we developed SC-003, an ADC consisting of a humanized monoclonal antibody linked to a PBD dimer via a cleavable linker. We show that SC-003 specifically binds to DPEP3-expressing cells and, upon internalization, elicits cytotoxicity via release of its PBD warhead following lysosomal degradation of the antibody component. A single dose of SC-003 induced tumor regression in DPEP3-positive HGSC PDX models, including platinum-resistant PDX models. Mechanistically, we show that the anti-tumor effect of SC-003 is mediated by a significant reduction in TIC frequency. Moreover, combination with an anti-PD1 antibody potentiated SC-003 efficacy in a syngeneic mouse model engineered to overexpress human DPEP3. In summary, these findings support the clinical development of SC-003 as a novel therapeutic agent for HGSC.

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CATALYTIC SUBUNITS SWITCH DRIVES RESISTANCE TO EZH2 INHIBITORS IN ARID1A-MUTATED CELLS

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The SWI/SNF chromatin remodeling complex is altered in ~20% of human cancers. ARID1A, a component of the SWI/SNF chromatin-remodeling complex, is the most frequently mutated epigenetic regulator in human cancers. Inactivation of the SWI/SNF complex is synthetically lethal with inhibition of EZH2 activity. EZH2 inhibitors are entering clinical trials for specific tumor types with SWI/SNF mutations. However, mechanisms of de novo or acquired resistance to EZH2 inhibitors in cancers with inactivating SWI/SNF mutations are unknown. Here we show that the switch of the SWI/SNF catalytic subunits from SMARCA4 to SMARCA2 drives resistance to EZH2 inhibitors in ARID1A-mutated ovarian cancer cells. SMARCA4 decease dominates over SMARCA2 increase in the switch. SMARCA4 loss leads to suppression of apoptotic pathways through upregulating anti-apoptotic genes such as BCL2 in the EZH2 inhibitor resistant cells. EZH2 inhibitor resistant ARID1A-mutated cells are hypersensitive to BCL2 inhibitors such as ABT263. ABT263 is sufficient to overcome resistance to EZH2 inhibitor and synergistic with EZH2 inhibitor in vivo in ARID1A-inactivted ovarian tumour mouse models. Together, these data establish that the switch of the SWI/SNF catalytic subunits from SMARCA4 to SMARCA2 underlies the acquired resistance to EZH2 inhibitors and BCL2 inhibition alone or in combination with EZH2 inhibition represents a novel strategy to overcome and/or prevent EZH2 inhibitor resistance in ARID1A-mutated cancers. Given that the SWI/SNF subunits are among the most frequently mutated genes in human cancers and EZH2 inhibitors are in the clinical trials for tumor with mutations in the SWI/SNF complex, we expect our findings to have far-reaching implications for developing cancer epigenetic therapeutics.

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PRE-EXISTENCE OF POLY-RESISTANT CANCER STEM CELLS IN HIGH-GRADE OVARIAN CANCER

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INTRODUCTION: High-grade ovarian cancer (HGOC) shows excellent responses to standard-of-care surgery and paclitaxel/carboplatin therapy only to relapse 6-24 months later with typically resistant disease. While the origin of this recurrent, resistant disease is unclear, most believe it is acquired by the action of chemotherapeutics. Using novel stem cell technology that enables the cloning of cancer stem cells (CSCs) from epithelial cancers, we have generated large libraries of CSCs from multiple cases of HGOC. And while the vast majority of these CSC clones are killed by standard-of-care chemotherapeutic drugs, a minor fraction shows profound resistance not only to paclitaxel/carboplatin but to a wide range of structurally unrelated chemotherapeutic drugs to which these cells had no prior exposure. We describe screens for drugs that selectively target this resistant CSC population.

METHODS: Libraries of 10- to 100,000 CSC clones were generated from individual, therapy naïve, HGOC resections using technology we developed for cloning so-called "adult" stem cells from normal columnar epithelia (Wang et al., 2015, Nature, 522, 173).

RESULTS: Paclitaxel/carboplatin resistant CSCs were identified in CSC libraries derived from therapy naïve tumors at ratios of 1:50 to 1:300. By copy number variation, these resistant variant clones proved distinct from the bulk of CSCs, and by gene expression analysis varied from sensitive clones by more than 700 differentially expressed genes. Independent resistant clones from the same library clustered with other resistant clones by both copy number variation and gene expression profiles, suggesting the possibility that resistance within a single tumor is dominated by a single type of resistant CSCs. Clones resistant to paclitaxel/carboplatin were screened in a 384-well format against a wide range of experimental drug-like molecules. These pre-existing resistant clones also proved to be profoundly resistant to a large number of structurally unrelated chemotherapeutic drugs. This same screening program identified drugs that act alone or in combination with paclitaxel to eliminate these resistant clones, suggesting a route to personalized medicine for addressing the problem of recurrent disease in HGOC.

CONCLUSIONS: Tumors from patients with HGOC possess clonogenic CSCs including variants that are resistant to a broad spectrum of chemotherapeutics to which they have not been exposed. It is likely that such CSCs would survive standard-of-care chemotherapy and contribute to the recurrent disease seen in HGOC. We have identified known and experimental drugs that specifically eliminate these resistant variants and the overall platform represents a potential strategy to addressing the problem of recurrent disease in these patients.

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TARGETING UNFOLDED PROTEIN RESPONSE FOR OVARIAN CANCER THERAPY

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Standard paclitaxel-platinum-based chemotherapies often lead to relapses and chemoresistant diseases. New therapeutic strategies are urgently needed for improving the clinical outcomes of ovarian caner patients. Our goal is to identify dysfunctional cellular pathways that are critical for tumor progression and drug resistance and to design novel therapeutic interventions that affect these altered cellular functions.

The dysregulation of unfolded protein response (UPR) pathway is often found in cancer cells and contributes to cancer cell survival and their resistance to stress caused by chemotherapies, hypoxia, and nutrition deprivation. The objective of our research is to develop new therapeutic agents to target UPR in ovarian cancer cells in order to overcome chemoresistance.

Sulfonamides (SFs) have been used to synthesize antibacterial drugs. We have recently discovered a family of new SFs with anticancer activity. Based on preliminary study, we hypothesize that these new SFs induce apoptosis in ovarian cancer cells through targeting UPR.

Using one of the SFs, namely SF-Y3, we compared its effects on epithelial ovarian cancer (EOC) cell lines and immortalized normal fallopian tube (FT) cell lines by performing luminescent CellTiter assay. Phospho-S6 ribosomal protein (P-S6) staining and Annexin V-FITC/PI staining assays were performed to evaluate the effects of SF-Y3 on cell health. Human transcriptome array (HTA) was used to identify the gene expression changes in SF-Y3-treated EOC cells, which results were confirmed by quantitative real-time PCR (qPCR). Western blot and XBP1 RNA splicing PCR were performed to assess the activation of proteins in UPR pathway. Using 4u8c, an inhibitor of the ER transmembrane protein IRE1, we determined whether inhibiting UPR could rescue cancer cells from the SF-Y3-induced apoptosis. Co-immunoprecipitation (co-PI) was used to determine the effects of SF-Y3 on the ER membrane protein-protein interaction. Moreover, we encapsulated SF-Y3 with nanoparticle to improve its bioavailability for evaluating the in vivo efficacy in EOC mouse model as a single treatment and in combination with platinum-based chemotherapy.

The cell viability data demonstrated that SF-Y3 significantly reduced the viability of EOC cells expressing high levels of Bip1, a key chaperone protein in the endoplasmic. SF-Y3 was less effective in EOC cells with low levels of Bip1 and has no effects on normal FT cells. P-S6 and Annexin V staining assays demonstrated that SF-Y3 inhibited EOC cell proliferation and induced apoptosis. HTA and qPCR data showed that the UPR genes were unregulated by SF-Y3. Western blot and XBP1 RNA splicing PCR results indicated that SF-Y3 activated proteins in the UPR pathway, including ATF6, PERK, eIF2, XBP1, and CHOP. SF-Y3 interrupted the interaction between Bip1 and three ER membrane-associated sensors, supporting that Bip1 is a possible target of SF-Y3. UPR inhibitor 4u8c partially rescued the apoptosis induced by SF-Y3. These data support that SF-Y3 has anticancer activity in EOC models possibly through inhibiting Bip1 and inducing UPR-induced apoptosis. Further investigation of how SFs interact with Bip1 and UPR pathway in vitro and in vivo may lead to new approaches to overcome drug resistance and a significant therapeutic advance for EOC.

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INHIBITION OF PARG, SENSITIZES OVARIAN CANCER CELLS TO PARP INHIBITORS AND DNA DAMAGING AGENTS

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BACKGROUND: Poly(ADP-ribose) glycohydrolase (PARG) is responsible poly(ADP-ribose) (PAR) catabolism, which is synthesized by poly(ADP-ribose) polymerases (PARPs) at the site of DNA single-strand breaks (SSB). Faulty PAR formation or disintegration inhibits SSB repair. PARP inhibitors (PARPi) exploit the synthetic lethality between SSB repair and homologous recombination (HR) double strand break (DSB) repair. The most effective PARPi traps PARP at the SSB site, causing a double strand break during DNA synthesis that the cell cannot repair. The trapping of PARP can also be achieved through PARG inhibition. Due to the high rate of HR defects in epithelial ovarian cancer, the aim of this study is to evaluate the effect of a PARG inhibitor (PARGi) on epithelial ovarian cancer cell lines, alone and in combination with PARPi or cisplatin.

METHODS: PARG protein levels were assessed in 74, unselected, snap-frozen, tumors kept in our bio-bank by western blotting and immuno-histochemistry (IHC), as well as in the TCGA database. PARG was knocked down in two cell lines resistant to PARP inhibition, OVCAR3 and SKOV3. The knockdowns were subjected to cell migration and cell proliferation assays, as well as single cell analysis. BRCA1 mutated and BRCA1 wild-type cell lines were exposed to clinically relevant doses of PARGi, PARPi, or cisplatin, separately or in various combination. Therapeutic efficacy was assessed using colony formation assay. Western Blotting was used to detect the levels of PARG and PARP proteins in the cell lines.

RESULTS: PARG mRNA was expressed in 30% of tumors in TCGA. PARG protein was also detected in 30% of the biobank tumors analyzed. PARG knockdowns exhibited reduced rates of cellular proliferation and significant G2/M cell cycle arrest. In PARG over expressed ovarian cancer cell lines, PARG inhibitor similarly reduced cell migration (OVCAR3 and SKOV3: 75% reduction, SNU-251: 48% reduction).

CONCLUSION: PARG inhibition appears to be a viable, complementary strategy to PARP inhibition in HR-deficient cancers. The effect of BRCA1 on the efficacy of PARG inhibition, as well as various treatment combinations, are currently under investigation.

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SEQUENTIAL THERAPEUTIC TARGETING OF OVARIAN CANCER HARBORING DYSFUNCTIONAL BRCA1

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BACKGROUND: Ovarian cancer is the most lethal gynecologic cancer. High grade serous ovarian cancer (HGSC) is the most common and deadly histological subtype. The current standard treatment protocol involves primary debulking surgery followed by platinum-based combination chemotherapy. PARP inhibitors(PARPi) are the first approved personalized treatments used in BRCA1-mutated recurrent ovarian cancer patients and have shown promising clinical results. Previously published data in collaboration with Dr. Witcher's lab, we described a significant reduction in PARP1 protein levels in patients after giving standard carboplatinum-paclitaxel chemotherapy that is effecting the clinical efficacy of PARP inhibitors in clinical trials. PARP inhibitors are currently administered after standard chemotherapy, when PARP levels are the lowest which was clearly shown in the previous published paper. Applying novel strategy and following the sequence of administration, giving PARP inhibitors first followed by standard chemotherapy might improve response rates. This study aims to evaluate this strategy (in vitro) in the pre-clinical models.

METHODS: BRCA1 mutated (UWB1.287, SNU-251), epigenetically silenced (OVCAR8), and wild-type BRCA1 (OVCAR3, SKOV3, A2780P & A2780R) cell lines were exposed to clinically relevant doses of PARPi, either followed by standard chemotherapy, or the inverse sequence. Therapeutic efficacy was assessed using colony formation assay. Apoptotic index was evaluated by cell cycle analysis and apoptotic assays using flow cytometry. Western Blotting was used to detect the levels of relevant apoptotic and cell cycle proteins.

RESULTS: Exposure to PARPi prior to standard chemotherapy sensitized BRCA1 mutated or epigenetically silenced BRCA1 cell lines to lower doses of Cisplatin (CP) or Paclitaxel (PT). Similarly, pre-treatment with PARPi prior to chemotherapy induced apoptosis more effectively in the same cell lines. Similar results were observed in BRCA1 wild-type cell lines and cell lines in which BRCA1 functionality was restored.

CONCLUSION: Pre-treatment of cell lines with PARPi followed by standard chemotherapy is more efficient (in vitro) in inhibiting growth and inducing apoptosis than the present sequence of chemotherapy followed by PARPi.

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TARGET DISCOVERY OF NATURAL PRODUCT INSPIRED PHYLLANTHUSMINS FOR TREATMENT OF HIGH GRADE SEROUS OVARIAN CANCER

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BACKGROUND: High grade serous ovarian cancer (HGSOC) is a lethal gynecological malignancy with a need for new therapeutic agents. Many of the most widely used chemotherapeutic drugs are either derived from or are semi-synthetic derivatives of natural products. We developed potent synthetic analogs (PHYs) of the phyllanthusmin class inspired by prior natural product isolated from Phyllanthus poilanei Beille.

MATERIALS & METHODS: HGSOC cell lines, OVCAR3 and OVCAR8, and non-tumorigenic controls, IOSE80 and FT33, were used in this study. Cytotoxicity assays included sulforhodamine B assay, and annexin X/PI staining and Western blotting for confirmation of apoptosis induction. A photo affinity labeling method was used to attach PHY analogs to solid phase support. Targets were isolated using a pulldown technique and mass spectrometry. CRISPR-Cas9 genome editing was used to knockout and confirm putative targets.

RESULTS: The most potent analog, PHY34, has nanomolar potency in HGSOC cell lines in vitro and displayed cytotoxic activity through late-stage autophagy inhibition and activation of apoptosis. PHY34 was readily bioavailable through intraperitoneal administration in vivo where it significantly reduced HGSOC tumor burden. Targets were identified using photo affinity labeling-aided protein pulldown and mass spectrometry, and confirmed by generating knockout cell lines of targets.

CONCLUSIONS: This class of compounds holds promise as a potential, novel chemotherapeutic approach and demonstrates the effectiveness of pleiotropically targeting autophagy and apoptosis as a viable strategy for combating high grade serous ovarian cancer.

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KNOCKOUT OF MTF1 RESULTS IN THE INHIBITION OF EMT IN OVARIAN CANCER CELLS

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Due to peritoneal metastasis and frequent recurrence, ovarian cancer has the highest mortality among gynecological cancers. Epithelial to mesenchymal transition (EMT) contributes to ovarian tumor metastasis. In this study, we report for the first time that metal regulatory transcription factor 1 (MTF1) was upregulated in ovarian cancer, and its high expression was associated with poor patient survival and disease relapse. Knockout of MTF1 using lentiviral CRISPR/Cas9 nickase vector-mediated gene editing inhibited EMT by upregulating epithelial cell markers E-cadherin and cytokeratin 7, and downregulating mesenchymal markers Snai2 and β-catenin in ovarian cancer SKOV3 and OVCAR3 cells. Loss of MTF1 reduced cell proliferation, migration, and invasion in both SKOV3 and OVCAR3 cells. Knockout of MTF1 upregulated the expression of the KLF4 transcription factor, and attenuated two cellular survival pathways, ERK1/2 and AKT. Our studies demonstrated that MTF1 plays an oncogenic role and contributes to ovarian tumor metastasis by promoting EMT. MTF1 may be a novel biomarker for early diagnosis as well as a drug target for clinical therapy.

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AN ANTI-AMPHIREGULIN ANTIBODY AS POTENTIAL TREATMENT FOR OVARIAN CANCER

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The ErbB family of receptors consists of four receptors, which bind with eleven ligands. These EGF-like ligands can be classified according to their affinity to EGFR: EGF, TGFα, HB-EGF and betacellulin are considered high affinity ligands, whereas AREG, EREG and EPGN are considered low affinity ligands. The literature reports that AREG is a type II cytokine which plays a role in inflammation, its expression is known to contribute to tissue repair and regeneration due to its ability to promote cell survival and proliferation. Furthermore, AREG is expressed by activated Th2 cells, mast cells, eosinophils, basophils and M1 macrophages.

Profiling the repertoire of EGF-like ligands in ascites fluids collected from 43 ovarian cancer patients, we found that 86% expressed high levels of AREG. These findings lead us to generate an anti-AREG antibody active against both human and murine AREG. Efficacy of this antibody was tested in several in vivo experiments using anti-AERG alone or in combination with chemotherapy (cisplatin). A murine anti-EGFR antibody was used as a positive control. Our results showed a significant prolongation of the lifespan of mice treated with an anti-AREG antibody, postponing the advancement of the disease compared to the control untreated group, or the groups treated with an anti-EGFR antibody or with chemotherapy only.

One of our next goals is to establish a model that is completely murine. We are using immunocompetent C57 black female mice, the ID8 cell line which is an ovarian cancer mouse cell line and our antibody, which blocks the murine form of AREG. We hope that in this way we would be able to evaluate the involvement of the immune system. The knowledge we will gain about the efficacy and toxicity of an anti-AREG approach may not be available from humanized models.

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Alphabetical Index
of Speakers, Poster Presenters & Authors

Speaker & Poster Presenter names in black. Contributing Author names in gray.

Alphabetical Index
of Speakers, Poster Presenters & Authors (Continued...)

Speaker & Poster Presenter names in black. Contributing Author names in gray.

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of Speakers, Poster Presenters & Authors (Continued...)

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