Future perspectives in ovarian cancer research

Stockholm 16-17 November 2022

Abstract book

CANCERFONDEN

Contents

Welcome

Dear colleagues, dear friends,

The Swedish Cancer Society has the immense honour and pleasure of welcoming you to Stockholm for the great event **"Future perspectives in ovarian cancer research"**.

We are happy to hereby present the Abstracts of this international conference.

The conference takes place 16-17 November 2022 at the Karolinska University Hospital, in the Sune Bergström Aula, Stockholm, Sweden. It will cover several aspects of ovarian cancer research, including basic, translational, clinical and epidemiological science, from experimental studies to palliative care. The overall aim is to highlight new, important findings in the field and point out the main challenges and possibilities for important advances in the future.

Leading international and national scientists in the field of ovarian cancer will present their research during three sessions, covering i) Risk factors and prevention; ii) Pre-clinical models for translational ovarian cancer research and iii) New treatment modalities.

The second day of the conference ends with a workshop with the intention to clarify the challenges in ovarian cancer research and identifying where the greatest opportunities for important progress are.

This book includes Abstracts from the invited speakers of the conference and the submitted Abstracts for the Poster Presentations.

In addition, short presentations of the Steering committee board members are presented.

Thank you for your participation, and once again a warm welcome to Stockholm!

Steering committe

Dahm Kähler, Pernilla
Associate Professor Dept. Obstetrics and Gynecology Sahlgrenska Academy at University of Gothenburg, Gothenburg • Sweden

Associate professor Pernilla Dahm Kähler became a specialist in Obstetrics and Gynecology in 1999 and was the first certified Swedish subspecialist in gyne-oncologic surgery in 2005 and finished her PhD in 2006. She is the Head of Gyne-Oncology surgery at the Dept of Obstetrics and Gynecology at Sahlgrenska University Hospital.

Research area: Dr Dahm Kähler has been working intensively with optimizing gynaecological cancer surgery including centralization of advanced ovarian cancer. She is the national registry holder for the Swedish Quality Register for Gynecological Cancer, a member of the Swedish gynecologic cancer group and board member of national expert referral groups in gynecologic cancer.

Hedenfalk, Ingrid
Professor • Sweden

Affiliation: Lund University, Department of Clinical Sciences Lund, Division of Oncology, Breast and Ovarian Cancer Genomics group

Research area: We are interested in elucidating the cell-of-origin, molecular landscapes and microenvironmental impact associated with malignant transformation, evolution and progression of different ovarian cancer subtypes. Tissue from women at risk or who have developed ovarian cancer is interrogated using e.g. next generation sequencing, multiplex immunohistochemistry and spatial profiling to explore the pathogenesis of cancer initiation, evolution and the effects of systemic treatment pressure to identify cell or subtype specific vulnerabilities which may be further exploited in future therapeutic development. Whole genome sequencing of cervical cytology samples is used for the development of a sensitive and specific DNA-based diagnostic assay for early detection of ovarian cancer.

Lethi, Kaisa
Professor and Group Leader • Sweden

Affiliations: 1) Norwegian University of Science and Technology, Department of Biomedical Laboratory Science 2) Karolinska Institutet, Department of Microbiology, Tumor and Cell Biology.

Research area: Context-dependent and general mechanisms of tumor invasion, metastasis, and drug resistance with major focus on cell signaling and tumor microenvironment pathways in ovarian cancer. Interest in developing techniques for primary ovarian cancer and stroma cell isolation, ex vivo culture, and biomechanically controlled extracellular matrix models. The group has established ovarian cancer organoids and orthotopic metastasis model and collaborates on different aspects of clinical ovarian cancer therapy development.

Sundfeldt, Karin
MD PhD Professor/Consultant • Sweden

Affiliation: Sahlgrenska Academy at Gothenburg University, Sahlgrenska Center for Cancer Research, Dep of Obstetrics and Gynecology and Sahlgrenska University Hospital, Gothenburg.

Research area: I have a research group at the Institute of Clinical Sciences, Sahlgrenska Academy at Gothenburg University, Sweden. The group specializes in the biology of epithelial ovarian cancer (OC) and our goal is to minimize deaths by detecting OC in curable stages. To accomplish this, we search for new biomarkers for screening and early detection of OC. We have performed several studies with discovery of potential single biomarkers and panels of biomarkers. Blood, ovarian cyst-fluid, cervical and endometrial liquid biopsies is used for discovery and validation studies. Exploring new diagnostic tools for early detection or screening for gynecologic cancer as well as validating promising findings in larger cohorts are just some of the group's current focus areas.

Åvall Lundqvist, Elisabeth
M.D., PhD • Sweden

Elisabeth Åvall-Lundqvist is a Clinical Gynecologic Oncologist and Professor in Clinical Oncology, at the Department of Clinical and Experimental Medicine, Linköping University in Sweden. She is also Medical Director of the Clinical Trial Unit and Senior consultant at the Department of Oncology, Linköping University Hospital. She is chair of the Swedish Gynecologic Cancer Group.

Research area: Dr Åvall Lundqvist focus on population-based outcome studies in gynecological cancer. Åvall Lundqvist has been an active PI in phase II-III trials for ovarian cancer trials and served as Director of the Gynecologic Cancer Intergroup as well as President of the Nordic Society of Gynecologic Oncology.

Björkhem-Bergman, Linda
MD, PhD, Docent (Assoc Professor) • Sweden

Senior Consultant in Palliative Medicine, Karolinska University Hospital and Docent at Karolinska Institutet.

Associate scientific secretary at the Swedish Cancer Society.

Research area: Palliative medicine with special interest in Vitamin D, immunology and symptom management in palliative cancer care.

Kärre, Klas
MD PhD, Professor Emeritus of Molecular Immunology, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm • Sweden

Chairperson, The Scientific Committee of The Swedish Cancer Society.

Research area: Cellular and molecular immunology, NK cells, tumor immunology and immunotherapy.

Zedenius, Jan
MD PhD, Professor of Surgery, Department of Molecular Medicine and Surgery, Karolinska Institutet • Sweden

Senior Consultant Department of Breast, Endocrine Tumors and Sarcoma, Karolinska University Hospital.

Associate Scientific Secretary, the Swedish Cancer Society.

Research area: Prognostic factors in thyroid cancer, adrenal and neuroendocrine tumors.

Poster presentations

1. Pre-clinical & translational

P.01

Characterization of a subset of tissue resident NK cells in ovarian cancer ascites with anti-tumor properties

Elin Bernson[1, 2], Oisín Huhn[2], Veronika Karlsson[1], Swetha Kannan[2], Maria Lycke[1], Karin Sundfeldt[1], Francesco Colucci[2]

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[2] University of Cambridge, Department of Obstetrics and Gynaecology, UK

Background: Ascites, developed along with tumor spread into the abdomen in patients with high grade serous ovarian cancer (HGSOC), represents a unique tumor microenvironment with presence of tumor cells and immune cells. Among the immune cells found in ascites are natural killer (NK) cells, that have an intrinsic potential to kill tumor cells. However, earlier studies have reported reduced function of these cells.

Objectives: We set out to deeply characterize the ascites NK (aNK) subsets, both phenotypically and functionally, to better understand how the tumor eradication potential may be targeted by an immunotherapeutic approach.

Method: aNK from chemo-naïve HGSOC patients were characterized using flow cytometry. To investigate their potential antitumor properties, we performed functional assays towards the ovarian cancer cell line OVCAR-3. The NK cell activity in mouse was measured in C57BL/6 mouse against the ovarian surface epithelial cell line ID8.

Results: Our data reveal the presence of an aNK subset with tissue-residency (TR) properties, with a phenotype characterized by $CD56^{bright}CD49a^{+}$ $CD103^{+}$. The vast majority of these cells expressed the inhibitory receptor $NKG2A^{+}$. Despite the phenotypic similarity to less mature NK cells, this subset of $NKG2A^{+}$ aNK cells was responsive towards the ovarian cancer cell line OVCAR-3.
Using a mouse model of ovarian cancer, we found that $NKG2A^{+}$ NK cells were more responsive than $NKG2A^{-}$ cells towards ID8 cells *in vitro*, as assessed by degranulation response. Moreover, the degranulation was inhibited by the expression of the NKG2A ligand Qa1b on target cells.

Conclusions: We have identified a large subset of tissue resident NK cells in ovarian cancer ascites that are highly positive for NKG2A and may hold a tumor eradicating potential. Current experiments with human cells and *in vivo* mouse models are testing the hypothesis that NKG2A blockade may improve immunotherapy of women with ovarian cancer.

P.02

Characterization of exome variants in endometriosis paired with later developed endometriosis-associated ovarian carcinomas

Anna Linder[1], Sofia Westbom-Fremer[2], Constantina Mateoiu[3], Amanda Olsson Widjaja[3], Srinivas Veerla[2], Benjamin Ulfenborg[4], Ingrid Hedenfalk[2], Karin Sundfeldt[1]

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[3] University of Gothenburg, Department of Pathology, Sweden
[4] University of Skövde, School of Bioscience, Sweden

Background: Endometriosis is a molecularly complex disease, affecting about 10% of females in reproductive age. Although being pathologically benign, it shares several traits with cancer, including the ability to metastasize, resist apoptosis, and induce angiogenesis. Epidemiological studies have shown that endometrioma is associated with an increased risk of developing endometriosis-associated ovarian carcinoma (EAOC), predominantly of clear-cell or endometrioid subtype.

Objectives: The aim of the present study was to assess the genetics of ovarian endometriosis (OE) and subsequent EAOC in an attempted to identify key events linking the two diseases.

Method: Eleven women with preceding ovarian endometriosis and later developed EAOC (clear-cell carcinoma n=4, and endometrioid n=7) was enrolled for paired-sample whole-exome sequencing. Blood or adjacent stromal tissue was used as somatic control. Findings were validated with immunohistochemistry and MSI analysis.

Results: Median time between OE and EAOC diagnosis was 9 years. The OE group displayed an overall high mutational burden, median 2.8 non-silent mutations per Mb (range 0.2–73.5). Data showed cosine similarities ≥0.5 to cancer-associated mutational signatures such as homologous recombination and mismatch repair deficiency. There was a significant correlation between high median variant allele frequency (VAF) in OE, and extended time to EAOC diagnosis. Eight OE samples harboured a non-silent mutation in at least one known cancer-associated gene, only *KRAS* was mutated in more than one sample (n=2). Copy number variation analysis showed large genomic aberrations, affecting several cancer-associated genes, including a gain of 16p13.3 with partial overlap in one of two subsequent clear-cell carcinomas. In EAOC, the most frequently mutated genes were *TP53* (n=5), *KRAS*, *PIK3CA* and *AFF3* (n=3). Paired-sample analysis showed that eight cases shared one or more non-silent mutation, none located in cancer-associated genes.

Conclusions: Paired endometriosis and ovarian carcinoma exhibited genetic similarities. Although known cancer-associated genetic aberrations were common in OE, they generally did not persist malignant transformation.

P.03

Chemotherapy sensing profile of high grade serous ovarian cancer at a single-cell resolution

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[4] Department of Biochemistry, University of Cambridge, United Kingdom.

Background: High-grade serous ovarian cancer (HGSOC) is the most common histological subtype accounting for 75% of epithelial types of ovarian cancer. Chemotherapy resistance in HGSOC is still a big challenge during clinical treatment. The regulatory mechanism of drug resistance in HGSOC is still poorly understood and urgently needed to improve clinical decision making and thereby HGSOC patients' survival.

Objectives: We are mapping HGSOC cells' transcriptional changes in response to drug concentration and treatment duration to identify transcriptional profiles associated with drug resistance and thereby suggest effective targets for therapeutic interventions.

Method: 96-well format was developed to treat cells with different combinations of drugs, drug dose and drug treatment time, followed by single-cell RNA seq (scRNA-seq) combined with cell hashing. Several clinical-related drugs and their combinations, three doses and two treatment durations were performed in two HGSOC cell lines (COV362 and Kuramochi).

Results: After sequencing the hashtags and quality control of the data, single cells were assigned to their original well. Response of HGSOC cells to drug treatments in our assay was confirmed by cell cycle phase analysis. Preliminary results show that although the effect of a single drug, e.g. Cisplatin, on cell cycle phase was as expected, treatment with some of the drug combinations have different cell cycle profile compared to the individual drugs.

Conclusions: We have successfully set up the workflow of scRNA-seq and cell hashing for HGSOC cells and generated a large data set which is suitable for probing changes in transcriptional profiles at the clonal level in response to drug treatment and their association with development of drug resistance.

P.04

Construction of a diagnostic tool for early detection of high-grade serous ovarian carcinoma in liquid biopsies

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Background: Ovarian carcinoma (OC) has the highest mortality among gynaecological cancers. This is associated with late-stage diagnosis due to absent or unspecific symptoms. The high-grade serous ovarian carcinoma (HGSC) accounts for over 60% of the OC associated deaths. The most frequent genetic aberrations in HGSC are mutations in the *TP53* gene, present in ~96% of the tumours. It has previously been suggested that mutations in OC circulating tumour DNA (ctDNA) could be used for diagnostic purposes. However, the need for diagnostic methods capable of detecting asymptomatic HGSC is unmet.

Objectives: The objectives were to design a screening-tool for early detection of HGSC and to assess clinical-specimen types for diagnostic purposes.

Method: Primary tumours, cystic- and ascites fluid, cervical- and uterine samples were analysed for *TP53* mutations in DNA from patients with HGSC (n=19). The simple, multiplexed, PCR-based barcoding of DNA for sensitive mutation detection using sequencing (SiMSen-seq) approach was applied to design the *TP53* panel. The panel amplified 17 regions covering 619 nucleotide positions of *TP53*. The presence of *TP53* variants in patient derived samples was assessed with NGS.

Results: The panel identified variants in 18/19 (95%) of the primary tumours, with a VAF ≥ 9% (range: 9-91). Preliminary data indicate that two of three paired samples obtained corresponding mutations between different specimens from the same patient. In total 3/3 (100%) cystic-, 3/12 (25%) cervical-, and 1/2 (50%) uterine samples showed mutation at a consensus read depth of 3 and a minimum VAF ≥ 0.1%.

Conclusions: These findings indicate that the detection of *TP53* mutations in non-invasive liquid biopsies is obtainable with SiMSen-seq. The evaluation of the HGSC diagnostic panel holds promise for future clinical applications as a tool for diagnostics.

P.05

Copy number signatures for early diagnosis of high-grade serous ovarian carcinoma

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Background: The detection of ovarian carcinoma-derived somatic mutations in cervical samples and uterine lavages in several studies since 2013, has brought hope for the development of new biomarkers for early detection. High-grade serous ovarian carcinoma (HGSC) is strongly dominated by copy number alterations (CNAs). These CNAs are the consequence of underlying mutational processes in HGSC.

Objectives: We interrogated CNAs from low coverage whole-genome sequencing data in HGSC tumors, plasma, endometrial biopsies, and cervical samples to explore if copy number signatures can be used as a biomarker for early detection of HGSC.

Method: 123 samples, including pre-diagnostic cervical samples, from 18 patients with HGSC were included. Four *BRCA* mutation carriers (40 samples) and seven benign controls (41 samples) were also included. Estimations of ploidy and tumor fraction, and thus calculation of absolute copy number, were optimized through a combination of the ACE, Rascal, and ichorCNA bioinformatic tools.

Results: We extracted six fundamental copy number features from 69 diagnostic and pre-diagnostic cervical samples from patients diagnosed with HGSC. The signatures were generated using mixture modelling and non-negative matrix factorization. Interestingly, when applying our signatures to tumor and other samples, we could observe that signatures predominant in tumor samples were also represented in some diagnostic as well as pre-diagnostic cervical samples, plasma and endometrial biopsies.

Conclusions: In this study we provide support for the concept of constructing cancer-derived copy number signatures from diagnostic and pre-diagnostic cervical samples from HGSC patients. Whether these sigatures can be used for early detection of HGSC should be validated in larger and prospective studies in high-risk populations.

P.06

Data-driven analysis of a validated risk-score for ovarian cancer identifies clinically distinct patterns during follow-up and treatment

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Background: Ovarian cancer is the 8th most common cancer among women, and due to late detection prognosis is poor with an overall 5-year survival rate of 30-50%. Novel biomarkers are needed to reduce diagnostic surgery and enable detection of early-stage cancer by population screening. We have previously developed a risk-score based on a 11-protein plasma protein assay to distinguish benign tumors (cysts) from malignant ovarian cancer in women with adnexal ovarian mass.

Objectives: To validate the 11-protein plasma protein assay in further independent cohorts and study performance of the assay by analysis of samples collected during treatment and clinical follow-up.

Method: Analysis of plasma samples using a cusotm design PEA for the 11 protein-plex with absolute qunatification.

Results: By analysis of two independent clinical cohorts we validated the assay's performance, with a sensitivity of 0.83/0.91 and specificity of 0.88/0.92 for distinguishing benign tumors from malignant ovarian tumors. The risk-score follows the clinical development and is dramatically reduced upon treatment, and again increases with relapse and cancer progression. Data-driven modeling of the risk-score patterns during a two-year follow-up after diagnosis identified four separate risk-score trajectories with strong links to clinical development and survival. A Cox proportional hazard regression analysis of 5-year survival shows that at time of diagnosis the risk-score is the second-strongest predictive variable of survival after tumor stage, whereas MUCIN-16 (CA-125) alone is not a significant predictive variable.

Conclusions: The robust performance of the biomarker assay across clinical cohorts and the correlation with clinical development, indicate its usefulness both in the diagnostic work-up of women with adnexal ovarian mass and for predicting their clinical course.

P.07

Estrogen receptor β as a biomarker in granulosa cell tumors

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Background: Granulosa cell tumors (GCTs) are rare ovarian tumors, accounting for only up to 5% of all cases. These tumors are classified into two subtypes, juvenile GCT which occurs in prepubescent girls or young women, and adult GCT which mainly affects peri- or postmenopausal women. Although GCTs differ from epithelial ovarian cancers in several aspects, these tumors are still treated as general epithelial ovarian cancers. While GCT patients generally have a good prognosis, GCTs have a tendency for late recurrence with a very high mortality rate (80%), highlighting the need for new therapeutic strategies. Estrogen receptor β (ERβ/ESR2) is highly expressed in GCT and is a potential biomarker and therapeutic target.

Objectives: In this study, we aimed to better understand the biology of GCTs and to investigate the role of ERβ.

Method: Using tissue microarrays of formalin-fixed paraffin-embedded GCTs (n=50) and non-tumor ovaries (n=16), we evaluated ERβ as a potential biomarker for GCTs using a highly validated antibody (PPZ0506). We also performed RNA-sequencing of fresh-frozen samples (n=6).

Results: We found that 90% of the GCT samples expressed ERβ, where 77% of adult GCTs had a high expression. The expression of ERβ in adult GCTs was also positively correlated with current clinical GCT markers. However, no correlation with the proliferation marker Ki-67 was seen in either juvenile or adult GCT. Studying the transcriptome of GCT we found enrichment for genes within extracellular matrix organization, type 1 interferon signaling pathway, cell adhesion, cell proliferation, and estrogen biosynthetic processes. We also found significant transcriptomic differences between the two subtypes, mainly linked to higher cell proliferation and estrogen response in juvenile GCTs. Moreover, investigating the different ERβ splice variants identified an upregulation of ERβ2 (ERβ_cx) and ERβ4 in the tumors.

Conclusions: With these results, we propose ERβ as a diagnostic biomarker for GCT and a potential therapeutic target.

P.08

FAP+ cancer-associated fibroblasts are associated with worse outcome in high-grade serous ovarian cancer with high CD8-postive T-cell infiltration

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Background: CD8+ T-cells has been shown to improve clinical outcome in ovarian cancer. Tumor biology studies have implied that fibroblasts act as negative regulators of immune cell function in cancer.

Objectives: To investigate the clinical relevance of fibroblast activation protein (FAP) + cells in high-grade serous ovarian cancer (HGSC) in relation to CD8 expression.

Method: FAP and CD8 expression was analyzed by immunohistochemistry in a discovery cohort of advanced HGSC (N=113) and correlated to overall survival (OS) and progression-free survival (PFS). The findings from the discovery cohort were assessed in a validation cohort of HGSC (N=121) and in public available datasets (TCGA and GSE9891).

Results: Previous knowledge that high density of CD8+ cells in HGSC is associated with longer OS was confirmed (HR 0.55; 95% CI 0.33-0.85; p=0.008). In the discovery cohort, high intensity of FAP was associated with shorter median PFS in HGSC with high density of CD8+ cells (11.4 versus 18.6 months) as compared to low intensity of FAP (HR 4.03, CI 95% 1.38-11.72, p=0.01). In the validation cohort, high intensity of FAP in HGSC with high density of CD8+ cells was associated with shorter OS (p=0.01). The results were consistent in uni- and multivariate regression analyses. The two gene-expression data confirmed the association between high FAP expression and poor outcome in the high CD8- group, with a shorter PFS in the TCGA dataset and shorter PFS and OS in the GSE9891 dataset. In contrast, in both clinical cohorts as well as in gene expression datasets, high intensity of FAP had no impact on PFS or OS in cases with low expression of CD8+.

Conclusions: FAP positive fibroblast-subset is associated with poor prognosis restricted to a CD8 high density group of HGSC. Targeting the immunosuppressive action of fibroblasts may enhance the known positive prognostic effect of CD8-cells in ovarian cancer.

P.09
Galectin-3 – an immune modulator in ovarian cancer?

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Background: Due to the poor prognosis for overall survival in ovarian carcinoma (OC), there is a significant need for novel treatment options for these patients. When the cancer metastasizes, this is often accompanied by accumulation of ascites. Tumor cells and immune cells, including neutrophils and NK cells, are found in the ascites creating a unique tumor microenvironment. Galectin-3, a mammalian lectin, may be found in inflammatory environments, where it can induce reactive oxygen species (ROS) release in activated neutrophils. This, together with a correlation between neutrophil ROS release and impaired NK cell functionality, incited us to investigate the impact of galectin-3 on neutrophil – NK cell interactions in OC.

Objectives: This study aims to obtain greater knowledge about the immunomodulatory properties of galectin-3 in OC, which could potentially be utilized to harness the immune system to improve or find new treatment options for OC patients.

Method: Galectin-3 concentration was evaluated in ascites and serum from high-grade serous carcinoma (HGSC) patients using ELISA. Neutrophil ROS release was measured with an isoluminol chemiluminescence system. By using flow cytometry, we monitored neutrophil activation status and NK cell cytotoxicity towards the ovarian tumor cell line OVCAR-3.

Results: Galectin-3 levels were significantly higher in HGSC ascites compared to paired serum. The presence of activated neutrophils in HGSC ascites and their ability to produce ROS were also confirmed. Furthermore, an initial experiment showed that galectin-3 treatment induced ROS release in neutrophils from HGSC ascites but not in blood neutrophils from the same patient.

Conclusions: Our results demonstrate that neutrophils present in OC ascites are activated and capable of ROS release. Moreover, the results imply that galectin-3 increase neutrophil ROS release in the tumor microenvironment in OC ascites. On-going *in vitro* experiments will test the hypothesis that neutrophil ROS release induced by galectin-3 affects the ability of NK cells to eradicate OC cells.

P.10

Intratumoral adaptive NK cells have recall responses towards autologous ovarian cancer cells and correlate with better tumor killings

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Background: Immunotherapy has been regarded as most promising strategy aiming to eradicate malignancies especially solid tumors. Adaptive NK cells (aNK cells) capable of establishing the immune memory against specific antigens can become an ideal alternative with the capacity of resistance to TME and novel activation process. Yet, it remains barely reported about the role of aNK cells in solid tumors.

Objectives: We sought to expand tumor infiltrating aNK cells and map the correlation between aNK cells and corresponding tumor killing, simultaneously, examine aNK cell memory formation and recall responses against tumor cells.

Method: We used the flow cytometry, immunofluorescence, ex vivo culture, transwell and bioinformatics methods to explore adaptive NK cells in ovarian cancer.

Results: We found that aNK cells expanded from solid tumor exhibited/displayed a strong tumor reactivity in form of degranulation and cytokine production, compared to conventional cells (cNK cells) when encountering the autologous primary tumor cells. Such reactivity correlated with augmented tumor killing. Interestingly, following coculture with dendritic cells (DCs) loaded with specific tumor lysates, aNK cells elicited recall/memory responses only against the tumor cells which were previously presented by the DCs. Furthermore, we found aNK cells at close proximity to tumor cells in ovarian tumor tissue sections compared to cNK cells that were located in the stroma. Indeed, compared to cNK cells in a transwell migration assay, aNK cells displayed a specific migration towards tumor cells whose antigens have earlier been presented through DCs. Later, data from the Cancer Genome Atlas (TCGA) were utilized to overlay our used aNK cells phenotype signature with the clinical responses. We found that high aNK cells gene signature is associated with improved survival in ovarian cancer patients.

Conclusions: According to our study, intratumoral aNK cells correlate with tumor killing based on its antigen-specific immune memory against ovarian cancer, evidenced by our in vitro experiments.

P.11

Investigation of the DNA profile and suitable preanalytical handling of cervical samples for use in liquid biopsy-based diagnostics

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Background: Ovarian carcinoma (OC) is the leading cause of death from gynecologic malignancies and strategies for earlier detection are urgently needed. Liquid biopsies are an emerging research field and previous studies have shown that OC-derived mutations can be detected in transvaginally obtained samples. Cervical samples are routinely collected as part of the nation-wide screening program for cervical cancer and provide a suitable specimen for cell-free tumor DNA analysis using ultrasensitive methods. However, little is known about the DNA profile of cervical samples.

Objectives: Herein, we aimed to evaluate the DNA profile of cervical samples. Furthermore, we wanted to evaluate if storage temperature of cells in ThinPrep™ PreservCyt™ solution or the use of a SpeedVac Concentrator during the initial steps of DNA extraction affects the DNA integrity.

Method: Fragmentation analysis of DNA from cultured cells and cervical samples was performed using a 4200 TapeStation. Cultured cells were harvested and ThinPrep™ PreservCyt™ solution was added followed by incubation for 40h at room temperature. Thereafter, DNA was extracted directly or after storage at 4°C, -20°C or -80°C. Prior to DNA extraction, the methanol from the ThinPrep™ PreservCyt™-based cell suspension was removed either by pelleting or evaporation. DNA integrity was evaluated using automated electrophoresis and qPCR (short and long fragment quantification).

Results: Cervical samples showed a distinct fragment of approximately 250 bp. The same peak appeared in DNA extracted from cultured cells regardless of preparation method. A slight decline in DNA integrity number (DIN) was observed in samples stored at 4°C, where a SpeedVac Concentrator was used in the extraction process. DNA integrity, evaluated with qPCR showed no difference between different handling or storage temperatures.

Conclusions: Generally, the DNA integrity of cells were not affected by storage temperature or by the use of a SpeedVac Concentrator. The observed fragment at 250 bp needs to be further investigated.

P.12

Molecular Subtype Stratification is Required to Realize Prognostic Effects of Immune Infiltration in Endometrioid Ovarian Carcinomas

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Background: Endometrioid ovarian carcinoma (ENOC) arise through malignant transformation of endometriosis epithelium. Molecular alteration in this endometrial epithelial tissue is believed to be the dominant driver influencing endometriotic and subsequent tumor microenvironments. The presence of tumor infiltrating lymphocytes (TIL), especially CD8+ cells, can be prognostic in cancer. While the immune tumor-microenvironment (iTME) of high-grade serous ovarian carcinomas has been studied in great detail, little is known about the iTME of ENOC. We recently established four prognostic molecular subtypes of ENOC, so-named POLEmut (favourable outcome), MMRd (intermediate outcome), p53abn (poor outcome) and a final intermediate outcome group with no specific molecular profile (NSMP). ENOC subtypes are distinct from other ovarian carcinomas but are highly similar in genomic profile and outcomes to endometrial carcinomas (EC) classified with the same parameters.

Objectives: In the present study we evaluated the influence the iTME has on ENOC molecular subtypes and compare it to endometrial carcinomas.

Method: Tissue microarrays were stained for various different immune cells. T-cell lineages (CD3/CD8 and CD25/FoxP3/CD8), B-cell lineages (CD79a/CD20), and macrophages (CD68/PDL1) were quantified by multiplex immunofluorescence.

Results: In our cohort of 210 cases, each molecular ENOC subtype population was representative with expected survival outcomes. iTME evaluation revealed high levels of immune infiltration in MMRd and POLEmut subset of ENOC, similar to trends observed previously for EC. Overall, immune infiltration is variably spread within tumor epithelium and stroma. Stratifying into TIL_{low} and TIL_{high} clusters, little prognostic value was observed when examining all ENOC (OS $p=0.377$). Subtype analysis suggested NSMP ENOC belonging to a high-level immune infiltration cluster, have superior outcomes (OS and DSS $p<0.05$).

Conclusions: These findings accentuate the similarity between ENOC and EC and provide further support for histotype-stratified research and clinical prioritization of ovarian cancers. Further research into the nature of the NSMP ENOC subgroup has to be done.

P.13
Molecular vulnerabilities in treatment-resistant ovarian cancer

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Background: Ovarian cancer is the most deadly gynecologic malignancy worldwide. This is a consequence of late diagnosis and intrinsic or acquired chemotherapy resistance. The first-line chemotherapy has remained carboplatin and paclitaxel for almost 30 years. Regrettably, more than 80% of patients eventually present chemotherapy resistance making the treatment inefficient. The mechanisms underlying the chemoresistance of ovarian cancer are poorly understood.
Objectives: To identify chromosomally integrated shRNAs that are enriched or depleted in treatment-resistant tumor cells compared to untreated tumor cells from an in vivo model in order to investigate molecular vulnerabilities in high-grade serous ovarian carcinoma that may reveal new therapeutic targets.

Method: We transduced four human ovarian cancer cell lines, OVCAR8, OVCAR4, OVCAR3, and OVSAHO, with a whole-genome shRNA library comprising >75,000 different shRNA clones, targeting >15,000 genes. The transduced cells were implanted intraperitoneally into immunocompromised NSG mice. The transplanted mice were treated with carboplatin and paclitaxel chemotherapy regimens similar to that used in humans. After three cycles of chemotherapy, the treatment-resistant metastases and control tumors were harvested. The relative frequencies of specific shRNAs will be analyzed via next-generation sequencing (NGS).

Results: Tumors were harvested from all mice. The untreated groups showed an important difference in tumor quantity and/or size, with a higher amount and larger tumor size compared to the treated mice. The untreated mice also showed overall worse clinical status with gastrointestinal complications, resulting in ascites and icterus tissues. These differences were observed for all four cell lines. Next, the sequencing will be performed and we hope to present initial conclusions soon.

Conclusions: These experiments will reveal the genetic determinants of ovarian cancer resistance to chemotherapy in vivo. The corresponding proteins will comprise candidate molecular targets for the development of novel treatments against ovarian cancer.

P.14

Real cellular composition of ovarian cancer spheroids: the detailed observational findings with multi-photon microscopy

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Background: More than 70% of epithelial ovarian cancer (EOC) patients are diagnosed with much ascites, which are full of EOC cells. Mesothelial cells are major component in ascites.

Objectives: We aimed to reveal the real component and characteristics of EOC spheroids.

Method: We have evaluated the existing form of EOC cells in ascites. We tried to find a suitable marker to divide mesothelial cells with EOC cells with various immunofluorescent staining. Whole EOC spheroids were observed by using multiphoton microscopy. Invasion ability to collagen or mesothelial monolayer were investigated. We also performed RNA sequencing to reveal the molecular mechanisms related to aggressiveness of mesothelial cells interaction with EOC cells.

Results: In malignant ascites, nearly 100% of EOC cells existed as spheroids, not single cells. HBME-1 was a most reliable marker to divide mesothelial cells with EOC cells. We revealed that almost all spheroids included HBME-1 positive, mesothelial cells using multiphoton microscopy. EOC cells rapidly generated strict spheroids with mesothelial cells compared to spheroids with EOC cells alone (fluorescence-area 320 vs 460um^2/24hrs, p=0.002). Spheroids composed with EOC and mesothelial cells rapidly invaded into collagen gels, although spheroids with only EOC cells did not (933 vs 312um/72hrs, p<0.001). Surprisingly, some spheroids showed that mesothelial cells invaded at first, then EOC cells followed. When closely looked at the invasive front from spheroids into mesothelial layer, mesothelial cells invaded much further from spheroids. On the other hand, EOC cells still stayed inside the border. RNA sequence revealed high level of TGF-β-related pathway and hypoxia were upregulated. We focused on FSCN1 and Myo10. Blocking FSCN1 or Myo10 in mesothelial cells showed decrease abilities of these aggressive features of invasion.

Conclusions: We revealed that non-malignant mesothelial cells were existed in EOC spheroid in malignant ascites. These hetero-cellular spheroids showed aggressive features of invasion and migration via TGF-β-FSCN1 axis.

P.15

Spatial Characterization of Fallopian Tubes from Germline BRCA Mutation Carriers

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Background: It has been known for decades that women with germline *BRCA* mutations have an increased risk of developing high-grade serous ovarian cancer (HGSOC). After frequent reports of fallopian tube lesions in women undergoing risk-reducing salpingo-oophorectomy (RRSO), the focus on finding the origin of HGSOC shifted from ovarian surface epithelium to fallopian tube epithelium (FTE) and it has since been established that HGSOC indeed can originate from the FTE. However, except for the involvement of *TP53* mutations, little is known about the early changes in the fallopian tubes leading to cancer development.

Objectives: We aimed to investigate the spatial gene expression landscape of normal fallopian tubes from women with an elevated risk of developing HGSOC and to build an in-house pipeline for data analyses.

Method: Archival fresh frozen tissue samples from 13 women with germline *BRCA1* or *BRCA2* mutations undergoing RRSO were obtained and used in the Visium Spatial Gene Expression assay (10x Genomics). The initial data processing was conducted by SpaceRanger and Loupe Browser (10x Genomics), and data integration of all samples was performed using Seurat v4 and the Harmony R package. Also, H&E images were used to annotate the main tissue component of each Visium cluster.

Results: A total of 12 clusters were identified across all samples from the integrated data, and the clusters corresponded well to the tissue component labels. Two clusters were enriched for FTE cells, seven for stromal cells, and three consisted of a mixture of cell types. Further data analyses, including differentially expressed genes and gene ontology enrichment are ongoing.

Conclusions: Through Visium spatial gene expression profiling, we can preserve the spatial information and perform integration with single-cell reference data, despite the Visium data not being of single cell resolution.

P.16
Targeting IRAK3 can enhance response to therapy in ovarian cancer

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Background: Ovarian cancer represents an unmet medical need for many patients and patient response to therapy is limited by the hostile tumor microenvironment. Resistance to therapy may alter the functions of immune cells and counteract the anti-tumor immune responses. Therefore, uncovering the molecular mechanisms that regulate the interaction between cancer cells and immune cells in ovarian cancer may provide new targets for enhancing immune responses. Negative regulatory molecule interleukin-1 receptor-associated kinase (IRAK) 3 which is predominantly expressed in monocytes/macrophages regulates immunological tolerance and resistance to therapy in the tumor microenvironment.

Objectives: IRAK3 function on immune regulation in ovarian cancer is investigated.

Method: We generated a knockout (KO) model to target IRAK3 gene using the CRISPR/Cas9 precise gene editing technology in primary human monocytes isolated from healthy donors. For *in vivo* studies, we employed a novel immunocompetent mouse model where 9839 bp of IRAK3 gene is deleted by CRISPR/Cas9. Mouse model for ovarian cancer was generated by intraperitoneally inoculation of ID8 cell line in WT and IRAK3 KO mice.

Results: Genetic deletion of IRAK3 in primary human monocytes and mouse bone marrow cells was confirmed with gene and protein analysis. IRAK3 deletion in human monocytes/macrophages and mouse bone marrow cells increased inflammatory cytokines and chemokines secretion in response to TLR agonists. IRAK3 deletion delayed tumor growth-associated body weight and promoted survival in ID8 ovarian cancer mouse model. MHC-II expression on myeloid cell subsets increased while suppression markers CD39 and CD73 expression on CD8+ T cells decreased in tumor-bearing IRAK3 KO mice. Additionally, according to the public dataset data obtained from ovarian cancer patient samples, poor prognosis was predicted with higher IRAK3 mRNA levels in patients after chemotherapy.

Conclusions: IRAK3 can be a key negative regulator protein that modifies function of myeloid cells. Targeting IRAK3 may promote therapy efficacy in ovarian cancer by inducing anti-tumor immune responses.

P.17

Wnt-binding receptors as potential targets for ovarian cancer therapy

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Background: Ovarian cancer (OC) is the second most lethal gynecological cancer in developed countries mainly due to the lack of predictive and prognostic tools as well as resistance occurring against the main treatment options, namely taxane- and platinum-based chemotherapy.

Objectives: Our studies focus on targeting the molecular events that drive OC progression, with particular attention to the role of the Wnt-binding receptors, which are crucial in developmental processes and embryogenesis but can re-activate in tumors and foster cancer cell stemness.

Method:

1. Functional assays with OC representative cell lines and patient-derived cell cultures (PDCs)
2. High-Content image Analysis (HCiA) of 3D models
3. Biochemical analyses aimed at the detection and characterization of intracellular signaling
4. Multiomics approaches leveraging (phospho-)proteomics and transcriptomics data
5. Drug Sensitivity and Resistance Testing (DSRT).

Results: We established that the Wnt-binding receptor protein tyrosine kinase 7 (PTK7) is highly expressed in OC, contributing to the downstream signaling events that lead to cancer progression, metastasis, and therapeutic resistance. We observed that in OC, PTK7 expression modulates cell adhesion and sustains epithelial-mesenchymal transition. Furthermore, our study suggests that targeting PTK7 with mAb cofetuzumab modulates drug sensitivity in OC cells, specifically enhancing the response to paclitaxel.

Conclusions: Our pre-clinical studies point towards novel treatments via targeting PTK7 for better clinical outcomes in OC.

2. Clinical research

P.18

Correlation between CD8, FoxP3 and PD-1 expressing lymphocytes and relation to outcome in primary and metastastatic high-grade serous carcinoma

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Background: There may be differences in immune environment between primary and metastatic sites in high-grade serous carcinoma (HGSC). High presence of CD8+ cytotoxic T cells as well as PD-1+ T lymphocytes is associated with better survival, at least in primary tumors. Whether FoxP3 expressing regulatory T cells are beneficial is controversial.

Objectives: To map the presence CD8+, PD-1+ and FoxP3+ lymphocytes, CD68+ macrophages and CD20+ B lymphocytes and their prognostic impact in primary HGSC and concurrent metastases.

Method: Immunohistochemistry (IHC) was used to characterize inflammatory cells within tumor epithelium on a population-based TMA containing 130 consecutive cases of advanced HGSC diagnosed between 2011 and 2015. Each case had four to eight cores from primary tumor (PT, n=119), peritoneal metastases (Pmet, n=116) and/or lymph node metastases (n=54) represented in the TMA. The core with the highest proportion of positive cells on each site was designated the hotspot for each marker.

Results: On a core-to-core comparison, there was modest correlation between markers, the strongest being between CD8 and PD-1 (Kendall´s Tau for PT 0.58 and Pmet 0.51). Correlations between all markers were strong in both PT and Pmet ($p<0.001$ to 0.044), except for CD8 and CD20 and PD-1 and CD68 in Pmets, where no correlations were found. Overall survival (OS) and progression-free survival (PFS) was longer in the CD8high PT ($p=0.018$ and $p=0.020$), CD8high Pmet ($p=0.022$ and $p=0.003$) and PD-1high Pmet ($p=0.003$ and $p<0.001$) groups. No prognostic impact was found for FoxP3.

Conclusions: Although further site-specific analyses on intratumoral CD8, PD-1 and FoxP3 expressing lymphocytes are pending, we conclude that there is strong correlation between these markers as well as with macrophages and B lymphocytes in both PTs and Pmets. High CD8 expression in both PTs and Pmets was associated with better outcome, whereas PD-1 expression was only prognostic in Pmets.

P.19

Effect of Vitamin D on pain and fatigue in patients with cancer in palliative care – results from the "Palliative-D" trial

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Background: Pain and fatigue are common symptoms in patients suffering from advanced cancer. Previous studies have shown an association between low vitamin D levels and increased opioid doses as well as severe fatigue in palliative care patients. Pain and fatigue are prominent symptoms in ovarian cancer patients and may reduce quality of life (QoL).

Objectives: The aim of the 'Palliative-D' study was to test the hypothesis that correction of vitamin D deficiency reduces opioid use and fatigue in cancer patients admitted to palliative care.

Method: A multicenter randomized, placebo-controlled, double-blind trial in three home-based palliative care facilities in Sweden was performed. Patients with advanced cancer and 25-hydroxyvitamin D < 50 nmol/L were randomized to vitamin D3 4000 IU/day or placebo for 12 weeks. The primary endpoint was the difference of long-acting opioid use (fentanyl ug/h) between the groups during 12 weeks, based on four time points. The secondary outcomes included change in fatigue and Quality of Life (QoL).

Results: 244 patients were randomized, and 150 patients completed the 12 weeks trial. Of the randomized patients, 10% suffered from gynecological cancers. The vitamin D-group had a significantly smaller increase of opioid doses compared to the placebo-group after 12 weeks; −6.7 µg fentanyl/h ($p<0.05$). This corresponds to 10 mg less morphine /day. Vitamin D-reduced fatigue assessed with ESAS was −1.1 points (on a 11-graded scale) after 12 weeks ($p<0.01$), a change that is considered as clinically significant. QoL did not differ significantly between the groups. The vitamin D treatment was well tolerated with no severe adverse events.

Conclusions: Correction of vitamin D deficiency is safe in patients with advanced cancer and may have positive effects on opioid use and fatigue. These findings deserve further studies and may be beneficial for patients with gynecological cancer.

P.20

Nanoparticle based glycovariants as biomarkers of ovarian carcinoma

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Background: Ovarian carcinoma is the most lethal gynaecological malignancy with a poor 5-year survival rate, which could be improved to 90% if diagnosed at an early-stage. Aberrant glycosylation is a universal feature of cancer cells and detection of cancer-related glycosylation patterns of proteins presents a promising approach for improved cancer detection.

Objectives: This study reports the discovery and evaluation of nanoparticle aided sensitive assays for glycovariants of MUC16 (CA125) and MUC1 (CA15-3) in a unique collection of 347 paired ovarian cyst fluids and serum samples. The sample collection was divided into a discovery cohort (n=75) and patient validation cohort (n=272). Three immunoassays for CA125, CA15-3 and HE4 were also included for comparisons.

Method: The glycovariant approach is based on the use of highly fluorescent europium-chelate dyed nanoparticles onto which glycan specific proteins are coated to provide the necessary binding strength and signal amplification to provide low detection limits, while maintaining the original glycan-structure specificity.

Results: In the cyst fluids, MUC1 glycovariants exhibited striking discriminations of the malignant and benign groups with detection rate more than 80% for postmenopausal cases (n=208). In the serum, MUC16 glycovariant showed the best diagnostic performances. The improvement in detection over CA125 and HE4 was more than 15% in the early-stage group. Increased sensitivity was also seen in the postmenopausal group, more than 15% over CA125 and more than 25% over HE4.

Conclusions: The highly improved specificity, excellent analytical sensitivity, and robustness of the nanoparticle assisted glycovariant assays carry great promise for early detection of ovarian carcinoma. The concept can be explored for additional biomarkers to further enhance detection efficiency of early lesions.

P.21

Surgical and hormonal safety of performing opportunistic salpingectomy for prevention of epithelial ovarian cancer

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Background: Opportunistic salpingectomy is a method to potentially prevent epithelial ovarian cancer (EOC), based on theories that some EOC develops from precursor lesions in the distal Fallopian tube instead of the ovary itself. There are concerns that extended surgery at hysterectomy and sterilization may increase the risk of complications and affect ovarian function, causing early onset of menopausal symptoms.

Objectives: To evaluate surgical and hormonal safety of performing opportunistic salpingectomy compared with leaving Fallopian tubes *in situ* at benign hysterectomy or sterilization. In a long-term perspective evaluate the effect of opportunistic salpingectomy on the risk of EOC.

Method: HOPPSA (Hysterectomy and OPPortunistic SAlpingectomy) and SALSTER (SALpingectomy for STERilization) are national register-based randomized controlled trials (R-RCTs) with non-inferiority design. Patients planned for elective gynaecological surgery, are screened for participation within the Swedish National Quality Register of Gynecological Surgery (GynOp). Informed consent, randomization, documentation of surgical procedure, and follow-up questionnaires are provided within GynOp. Primary outcomes are complications to surgery and ovarian function. Long-term follow-up of EOC incidence is based on cross-linking with national registers.

Results: HOPPSA started recruitment in 2017 and SALSTER in 2019. Interim analyses have been conducted for both trials by an independent safety committee, resulting in recommendations to continue the trials as planned. By June 30th, 2022, HOPPSA had randomized 2076 women at 42 clinics and SALSTER 848. Recruitment to analyse complications in HOPPSA (n=2800) are estimated to be completed by the end of 2023 and recruitment to SALSTER (n=968) during autumn 2022.

Conclusions: Results regarding safety are anticipated during 2023 and the years ahead. Safety data will enable the writing of evidence-based guidelines to support women in their decision of removing healthy tubes. If a recommendation of opportunistic salpingectomy is feasible, based on no safety issues being present, the added procedure may decrease the incidence of EOC.

P.22

The role of computed tomography in the assessment of tumour extent and the risk of residual disease after upfront surgery in advanced ovarian cancer

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Background: Epithelial ovarian cancer is the gynaecological malignancy with the highest mortality rate, with a five-year survival rate below 45%. The standard treatment for AOC is primary debulking surgery (PDS) followed by platinum-based postoperative chemotherapy. Since surgery is the only way to improve prognosis for patients with AOC, the abdominal tumour extent must be well characterized to effectively plan the surgery and achieve maximal radicality.

Objectives: This study aimed to determine whether the peritoneal cancer index (PCI), the amount of ascites, and the presence of cardiophrenic nodes (CPLNs) visualized by computed tomography (CT) can assess the tumour extent (S-PCI) and residual disease (RD) for advanced ovarian cancer (AOC) patients treated with upfront surgery.

Method: In total, 118 AOC cases were included between January 2016 and December 2018 at Skåne University Hospital, Lund, Sweden. Linear regression and interclass correlation (ICC) analyses were used to determine the relationship between CT-PCI and S-PCI. The patients were stratified in complete cytoreductive surgery (CCS) with no RD or to non-CCS with RD of any size. The amount of ascites on CT (CT-ascites), CA-125 and the presence of radiological enlarged CPLNs (CT-CPLN) were analysed to evaluate their impact on estimating RD.

Results: CT-PCI correlated well with S-PCI (0.397; 95% CI 0.252-0.541; $p < 0.001$). The risk of RD was also related to CT-PCI (OR 1.069 (1.009-1.131), $p < 0.023$) with a cut-off of 21 for CT-PCI (0.715, $p = 0.000$). The sensitivity, specificity, positive predictive value and negative predictive value were 58.5, 70.3, 52.2 and 75.4%, respectively. CT-ascites above 1000 ml predicted RD (OR 3.510 (1.298-9.491) $p < 0.013$).

Conclusions: CT is a reliable tool to assess the extent of the disease in advanced ovarian cancer. Higher CT-PCI scores and large volumes of ascites estimated on CT predicted RD of any size.

P.23

Tumor Treating Fields - A Breakthrough Device for Ovarian Cancer Treatment

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Background: Tumor treating fields (TTFields) are portable, battery operated devices that deliver alternating electric fields via insulated electrode arrays to a tumor. Alternating electric fields have been shown to have an antiproliferative effect on various tumors. TTFields tuned to 200 kHz have been approved by the US Food and Drug Administration for treatment of glioblastoma. Dosimetric and modeling studies have shown that TTFields can penetrate the pelvis and abdomen. Preclinical trials have shown that the optimal frequency for tumor control in ovarian cancer is also 200 kHz.TTFields have also been shown to act synergistically with taxanes.

Objectives: We aimed to summarize the reported outcomes and safey of TTFields combined with weekly paclitaxel in treatment of ovarian cancer. Outcomes measured included overall survival, progression-free survival, time to undisputable deterioration in health-related quality of life (HRQoL), severity and frequency of adverse events.

Method: We conducted a systematic review of studies published in PubMed, Medline, and Embase reporting safety, overall survival, progression-free survival and response rate.

Results: Our search yielded 11 studies and 6 met inclusion criteria. Median age was 57 (range 45-78). All patient received prior platinum-based chemotherapy. Across studies, partial response rate was greater than 20%, clinical benefit rate was greater than 66%, and aggregate median progression free survival was 9 months.

Conclusions: TTFields concomitant with weekly paclitaxel are a breakthrough device with promise in multi-modal treatment of ovarian cancer. TTFields are associated with equivocal survival outcomes and low rates of treatment-related side effects. Results of an ongoing phase III clinical trial will further inform clinical practice. Oncologists in Sweden and around the world will benefit from a timely update on this emerging treatment for ovarian cancer.

P.24

Two new glycoform assays of CA125 and CA15-3 in the diagnosis of ovarian carcinoma

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Background: Epithelial ovarian cancer (EOC) is often diagnosed at a late stage. Detection of cancer specific glycosylation patterns of cancer antigens is a promising approach to improve early-stage detection and prolong survival.

Objectives: To find biomarkers for early detection of EOC using nanoparticle aided sensitive assays for two glycovariants of CA125 and CA15-3 in serum samples drawn at diagnosis of an ovarian mass.

Method: The cohort consists of 1475 women undergoing surgery in Umeå (n=535), Gothenburg (n=501) and Turku (n=439). Serum samples were analysed using two glycoform assays- recombinant human macrophage galactose-type lectin (MGL) and Sialyl-Thomsen-nouveau (STn) antibody of CA125 and CA15-3 as well as CA125, CA15-3 and HE4 enzyme immune assay (EIA). The glycoform assays are based on the use of highly fluorescent europium-chelate dyed nanoparticles onto which glycan specific proteins are coated to provide the necessary binding strength and maintaining the original glycan-structure specificity. Receiver operating characteristics (ROC) were applied. Area under the curve (AUC) and sensitivity at high (95%) specificity was compared.

Results: The cohort included 619 EOC, 120 borderline tumours and 737 benign ovarian cysts. There were 1093 postmenopausal and 380 premenopausal women. The sensitivity at high specificity for EOC vs benign tumors increased from 0.80 for HE4, the single best marker, to 0.87 combining HE4 with CA125STn or CA15-3STn. In stage I-II sensitivity increased from 0.64 to 0.67 (CA125STn+HE4) and 0.72 (CA15-3STn+HE4), and for stage III-IV from 0.86 to 0.95 and 0.94 respectively. The above-mentioned combinations also have the highest AUC compared with all other biomarkers.

Conclusions: This study suggests that the STn glycoform assays of CA125 and CA15-3 in combination with conventional HE4 detect EOC better than the conventional CA125, CA15-3 and HE4. These results indicate that the STn glycoform assays may play a role in improved early detection of EOC.

Invited speaker abstracts

S.01

Chimeric antigen receptor T cell therapy for the treatment of advanced ovarian cancer

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Background: Improvements in chimeric antigen receptor (CAR) T cell therapy for treatment of solid tumors are needed. The glycoprotein mesothelin (MSLN) has a low expression on normal cells but an increased expression in several solid tumors including ovarian cancer making MSLN an attractive target for antitumor therapy.

Objectives: To evaluate the antitumor efficacy of MSLN-directed CAR T cells against human ovarian cancer cells.

Method: We evaluated and compared the antitumor efficacy of second generation MSLN CAR constructs in vitro and in vivo using preclinical models of ovarian cancer.

Results: In vitro analysis of two second generation MSLN CAR constructs containing either the CD28 or the 4-1BB co-stimulatory domains (M28z CAR and MBBz CAR, respectively) showed superior antitumor efficacy of M28z CAR T cells as compared to MBBz CAR T cells. Our study also demonstrated that the loss of MSLN antigen by tumor cells and acquisition of MSLN on the cell surface of MSLN CAR T cells via trogocytosis resulting in fratricide killing of MSLN CAR T cells.
We established orthotopic mouse models of ovarian cancer and showed that treatment with M28z and MBBz CAR T cells significantly prolonged survival. However, tumor-infiltrating M28z and MBBz CAR T cells upregulated the immune checkpoints PD-1 and LAG3 in an antigen-dependent manner while MSLN-tumor cells expressed the corresponding ligands demonstrating that co-inhibitory pathways impede CAR T-cell persistence in the ovarian tumor microenvironment.
A novel MSLN (M1XX) CAR construct was evaluated in vivo and showed superior antitumor potency and persistence, together with a self-renewal gene signature and less exhausted phenotype.

Conclusions: MLSN CAR T cells can provide antitumor immunity against ovarian cancer however combination therapies may be needed to counteract the immunosuppressive tumor microenvironment.

S.02

Cognition after Bilateral Salpingo Oophorectomy (CABSOE): Considering the whole-body in cancer prevention

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Background: Women with the breast cancer gene mutations, BRCA1& 2 are counselled to have bilateral salpingo-oophorectomy (BSO) between ages 35-40. While BSO reduces breast and ovarian cancer risk significantly, it also eliminates ovarian production of 17-b-estradiol (E2), a steroid hormone that acts on every body system. The other body system corollaries of this reduction are rarely considered. From earlier studies, we knew that E2-depletion in young women significantly decreased verbal episodic and associative memory.

Objectives: We therefore asked whether BSO in BRCA1/2 women affected cognition and brain and determined whether:

1. BSO reduced verbal episodic and spatial working memory
2. Brain regions associated with this memory were altered
3. Taking estradiol replacement therapy ameliorated any changes
4. Those with BRCA/BSO felt they had been informed of the whole-body surgical consequences

Method: In collaboration with the University of Toronto, the Linköping University group (including the Faculty of Medicine and Gender Studies; CABSOE) recruited women prior to and from 1-10 years post-BSO. We administered neuropsychological tests, brain scans (structural/functional), measured hormone and cytokine levels, cortisol levels in hair, polysomnography, and determined the frequency of an Alzheimer disease genetic risk factor, APOE4. We also interviewed participants about their bodily changes & health care experiences.

Results: Women with BSO had decreased verbal episodic and working memory performance compared to age-matched controls. They also had decreased hippocampal volumes in CA23DG. Estradiol therapy ameliorated working memory and hippocampal changes. When asked about their experiences with BSO, Swedish women said, "It's difficult to imagine what it is like if you haven't been through it yourself" citing problems with a fragmented health care system and lack of information about the whole-body outcomes of BSO.

Conclusion: There are whole-body effects of risk-reducing BSO beyond the reproductive system. This needs to be shared since women with BSO do not feel fully informed about these corollary effects. While knowing that other body systems are affected by BSO would not change their decision to have BSO, they would have liked to have been more fully informed prior to BSO.

S.03
Co-targeting of HER2 and EpCAM using novel types of targeting probes for theranostics of ovarian cancer

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Background: Targeted delivery of radionuclides and drugs selectively to tumors while sparing healthy tissues might improve efficacy and safety of treatment of disseminated ovarian cancer (OC). Two potential targets in OC are human epidermal growth factor receptor 2 (HER2) and epithelial cell adhesion molecule (EpCAM), overexpressed in ca. 30% and ca. 70% of OCs. Radionuclide molecular imaging of expression of targets would enable stratification of patients to such therapies or their combination. A new class of targeting agents, engineered scaffold proteins, offer several advantages compared to the traditional monoclonal antibodies. Their small size allows reaching high contrast of diagnostic imaging. The possibility of precise modification allows attachment of cytotoxic drugs or radiolabels in a controlled way providing uniform agents with well-defined pharmacokinetics and toxicity profile.

Objectives: The goal was to investigate the feasibility of (co)targeting HER2 and EpCAM in OC for the purposes of radionuclide molecular imaging and targeted cytotoxic therapy.

Method: Novel engineered scaffold proteins (affibody, ADAPTs and DARPins) were evaluated as HER2- and EpCAM-targeting agents in OC xenografts. Probes for imaging of HER2 and EpCAM were preclinically evaluated and tested in Phase I clinical studies. Drugs, toxins, and radionuclides were preclinically evaluated as payloads. Factors influencing the efficacy of targeted therapy (nature of targeting moiety, molecular design, biodistribution and tumor-targeting properties) were investigated in vitro and in vivo. The feasibility of co-targeting HER2 and EpCAM was investigated.

Results: The tested constructs demonstrated their capacity to extend survival of mice bearing OC xenografts. Combination of EpCAM and HER2-targeting showed an additive effect without additional toxicity. Two imaging probes have demonstrated excellent imaging of HER2-expressing tumors in clinics. Valuable information about structure-property relationship and pharmacokinetic profile of a novel class of targeting agents was obtained.

Conclusions: This multidisciplinary translational project could aid the development of more effective and safe therapies.

S.04

Fellowship in ovarian cancer research – Towards Curing Ovarian Carcinoma with Targeted Alpha Therapy

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Background: Targeted alpha therapy is an emerging approach for treating micro metastasis of disseminated cancer. For this purpose astatine-211 is one of the most promising alpha particle emitting nuclides with a 7.2 h half-life and cyclotron production from natural bismuth. Astatine-211 require a tumor specific vector and antibodies constitute suitable carrier properties for targeting different malignancies.

Objectives: For ovarian cancer patients with intraperitoneal dissemination more than half of the patients will experience a relapse within two years, mainly in the abdominal cavity, which is why novel therapies that may prevent intra-abdominal relapse are desired. The overall goal of this project is to combine an antibody with astatine-211 in order to treat intraperitoneally disseminated ovarian cancer with targeted alpha therapy.

Method: To reach the objectives of this fellowship, two different aims have been explored. The first aim has focused on facilitating production and quality control of astatinated antibodies for use in a clinical phase I trial on ovarian cancer treatment. The second Aim concerns automation of astatine recovery and synthesis in order to facilitate multicenter clinical trials and routine clincal use of astatinated radiopharmaceuticals.

Results: Preparations for a clinical phase I trial in intraperitoneal treatment of disseminated ovarian cancer has e.g. resulted in an approved manufacturers permit for production of astatinated antibodies at the Radiopharmaceutical center at Sahlgrenska.
A commercial product has been created to recover astatine-211 from irradiated cyclotron targets and subsequently synthesize an astatinated radiopharmaceutical. This will significantly help in translating early phase clinical trial results with astatine into clinic practice and one such unit is now available at Sahlgrenska.

Conclusions: Significant steps have been taken towards realizing clinical trials with astatinated antibodies for intraperitoneal treatment of disseminated ovarian cancer and to translate these results to the clinic by development of automation hardware

S.05

Immunogenomic profiling for precision oncology in ovarian cancer

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Background: The majority of high-grade serous ovarian cancers (HGSCs) are deficient in homologous recombination (HR) DNA repair, most commonly due to mutations or hypermethylation of the BRCA1/2 genes. However, the underlying genotypic driver is still unknown in 30-40% of the tumors, and the variability of the molecular phenotypes and clinical behavior of the HGSC genotypes has hampered our understanding of the disease and the development of more effective therapeutic strategies. We recently developed an optimised test to reliably stratify HRD in HGSCs using somatic allelic imbalances (**Perez et al. Biorxiv 2021**).

Objectives: In recent years it has become increasingly evident that the anti-tumor immunity plays a critical role in HGSC therapy responses and clinical outcomes. Interestingly, emerging evidence suggests that HGSC genotypes harbor distinct immune escape mechanisms; BRCA1/2 deficient HGSCs have been associated with a higher neoantigen load and immune cell infiltration (Strickland 2016). A more detailed understanding of the spatial interplay of tumor genotypes and immune microenvironment is needed to develop more efficient immunotherapeutic strategies for HGSC.

Results: Recently, we discovered how the BRCA1/2 mutations shape the single-cell phenotypes and spatial interactions of the tumor microenvironment. (**Launonen et al, Nature Communiations 2022**). Using a highly multiplex imaging and bioinformatics we generated spatial proteomic from 124,623 single cells from 112 tumor cores originating from 31 tumors with BRCA1/2 mutation (BRCA1/2mut), and from 13 tumors without alterations in HR genes. We identified a phenotypically distinct single-cell tumor microenvironment with evidence of increased spatial immunosurveillance in the BRCA1/2mut tumors. Importantly, we showed that a proliferative tumor-cell subpopulation has a prognostic role, which associated with enhanced spatial tumor-immune interactions by CD8+ and CD4 + T-cells in the BRCA1/2mut tumors. By contrast, the immune landscapes were dictated by the stromal cell subpopulations, with less tumor-immune interactions suggestive of stromal barriers in the HRwt tumors.

S.06
Modeling the Origins of Ovarian Cancer and the Impact of a Novel Tumor Microenvironment Component

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One of the main challenges currently faced in ovarian cancer treatment is that the primary platinum/taxane therapy used to treat patients does not provide durable responses or meaningfully extend survival. The overall survival for ovarian cancer patients with advanced disease is less than 30% with most patients recurring within 5 years of initial therapy. Once resistant disease develops there are few second line therapy options, which inevitably leads to disease progression. Thus, there is a critical need to identify new therapies that can overcome ovarian cancer chemoresistance and produce meaningful increases in patient survival. In recent years, new efforts have focused on targeting the tumor microenvironment (TME), the complex mixture of non-cancer cells that surround and support the cancer cells within the tumor. Different strategies have evolved to target the TME, such as immune checkpoint inhibitors or angiogenesis inhibitors. However, these TME targeting therapies have limited effect when used to treat ovarian cancer patients. Most ovarian tumors have a microenvironment that is highly immunosuppressive and do not respond or are resistant to angiogenesis inhibitors. Thus, new TME targeting therapies must be developed that can induce a therapeutic effect in ovarian tumors and can be used to treat most ovarian cancer patients. A novel TME component ripe for therapeutic targeting is the nerve fibers that infiltrate tumors in a process termed tumor innervation. Recent studies in several cancers have shown that tumor innervation can promote tumor growth and metastasis. However, in ovarian cancer the role of innervation in promoting cancer progression remains undefined. Here we show that Transient Receptor Potential cation channel subfamily V member 1 (TRPV1)+ sensory innervation plays a significant role in driving ovarian cancer growth and metastasis. Analysis of patient samples shows that sensory innervation is much higher in ovarian tumors vs benign reproductive tissues. These nerve fibers can be traced back to the thoracic spinal dorsal root ganglia in mouse models and electrophysiologic activity can be measured in highly innervated human tumors. In addition, ablation of TRPV1 sensory nerve in vivo causes markedly reduced tumor burden and prolongs survival in a syngeneic mouse model of ovarian cancer metastasis. Taken together, our results establish TRPV1+ sensory innervation as a novel driver of ovarian cancer growth/metastasis and a potential therapeutic target for ovarian cancer treatment.

S.07
Molecular and clinical heterogeneity of high-grade serous ovarian cancer

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High-grade serous ovarian carcinomas HGSC have highly complex genomic profiles with few targetable mutations. This marked chromosomal instability (CIN) has impeded molecular classification and the development of precision medicine approaches. We hypothesised that patterns of copy number aberrations might specifically identify different mutational processes on the HGSC genome. We have developed copy number signatures that provide a systematic framework to comprehensively characterize the diversity, extent and origins of CIN and are carrying out pre-clinical and clinical studies that indicate how copy number signatures can provide disease stratification and treatment prediction in HGSC.

S.08
Mouse models for BRCA1 associated tumorigenesis

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Background: Genetically engineered mouse models (GEMMs) and patient-derived xenograft (PDX) models of human cancer not only permit us to gain a detailed insight into the specific genetic changes that drive tumor development and metastasis but also provide powerful tools to study the mechanisms underlying drug response and acquired resistance. Once these processes are understood in sufficient detail it may be possible to design combination therapies that not only cause complete remissions but also eliminate remnant cells that elicit recurrent disease.

We have generated multiple GEMMs and PDX models of BRCA1-associated triple-negative breast cancer (TNBC). The BRCA1-deficient mammary tumors from these mouse models are characterized by genomic instability and hypersensitivity to DNA-damaging agents, including platinum drugs and PARP inhibitors (PARPi). Nevertheless, none of these drugs are curative: tumors grow back after drug treatment and eventually become resistant. We found that PARPi-resistance of BRCA1-deficient GEMM tumors can be induced by several mechanisms, including activation of drug efflux transporters, type of *BRCA1* mutation, and loss of components of the 53BP1-RIF1-shieldin and CST complexes that govern end-protection of DNA double-strand breaks.

We have also developed several GEMMs for somatic modeling of BRCA1-mutated TNBC using intraductal injection of lentiviral vectors for stable overexpression of exogenous genes and CAS9-mediated disruption or APOBEC-CAS9-mediated base editing of endogenous genes. We have used these GEMMs to validate RB, PTEN, PIK3CA, MYC and MCL1 as bona fide driver genes in BRCA1-associated breast cancer. Moreover, MCL1 inhibition potentiated the in vivo efficacy of the PARPi olaparib, underscoring the therapeutic potential of this combination for treatment of BRCA1-associated cancer patients with poor response to PARPi monotherapy.

S.09
Organoid-based models for precision oncology and research

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Background: Few new therapies for treating patients with high-grade serous ovarian carcinoma have reached the clinic in recent decades. Challenges include the genetic and phenotypic complexity of the disease as well as a sparsity of relevant model systems. In vitro investigations have been limited by a lack of primary cell culture models. Short-term primary cell culture models are easily established, but the success rate of establishing robust long-term cultures that allow for drug resistance studies or genetic manipulation have historically been poor and very few models are available for the scientific community.

Objectives: To address this challenge, we developed an improved organoid establishment and culturing protocol for high-grade serous ovarian carcinoma cells.

Method: We tested more than 50 different supplements and growth factors and whether they could promote long term growth over several passages.

Results: The testing led us to two different medium formulations, one rich in supplements and stimulants and one with minimal stimulant additions. Some samples grow well in the former and aome in the latter. This culturing protocol has allowed us to establish stable, long-term growing organoid cultures with a success rate of 50% from cryopreserved patient material. The cultures consist of 100% cancer cells and are matching the original tumor cells in terms of genetics and phenotypes.

Conclusions: The culture models allow us to perform drug resistance studies, lineage tracing of the cells, genetic editing and fine-tuned co-culture models. Importantly, we are now also making as many as possible of these available for the scientific community through a public biobank.

S.10
Prevention in hereditary and non-hereditary ovarian cancer

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Background: Ovarian Cancer (OC) affects 314,000 women annually, but presents at advanced stages and has high mortality (207,000 deaths/year). Screening for ovarian cancer has not been found to have a moratlity impact and national screening programmes are not available. Up-to 20% of OC cases are due to pathogenic variants (PVs) in OC Cancer Susceptibility Genes (CSGs) and are potentially preventable. Additionally, complex risk-algorithms incorporating genetic (CSGs and polygenic-risk-score (PRS)) along-with non-genetic (family history (FH)/epidemiologic/reproductive factors) variables are now available and can provide personalised risk prediction for OC risk. Increasing awareness and acceptability of genetic-testing, falling genetic-testing costs, coupled-with changes in clinical practice including increasing genetic-testing at cancer diagnosis and recent calls for population-testing are leading to ever-increasing identification of unaffected women at increased OC risk. Risk reducing salpingo-oohorectomy is the most efective method of preventing ovarian cancer and is now recommended in women over a 4-5% lifetime risk of OC, thus broadening access for precision prevention. Risk reducing early salpingectomy and delayed oophorectomy is an emerging strategy which can provide a level of risk reduction while avoiding the detrimental impacts of early menopause. Opportunistic salpingectomy is now recommended at routine gynaecological surgery. Uncertainty remains around the precision of the level of risk reduction associated with salpingectomy. Contraceptive pill use is associated with a reduction in OC risk and there are emerging data demonstrating an association with aspirin.

Objectives: To present an overview of current ascertainment and prevention strategies for women with hereditary and non-hereditary OC

Method: The lecture will present an overview of current ascertainment and prevention strategies for women with hereditary and non-hereditary OC.

S.11
Proximal liquid biopsy for early detection of ovarian cancer

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No current screening methods for high-grade ovarian cancer (HGOC) guarantee effective early detection for high-risk women such as germline BRCA mutation carriers. Therefore, the standard-of-care remains risk-reducing salpingo-oophorectomy (RRSO) around age 40. Proximal liquid biopsy is a promising source of biomarkers, but sensitivity has not yet qualified for clinical implementation. We aimed to develop a proteomic assay based on proximal liquid biopsy, as a decision support tool for monitoring high-risk population. Ninety Israeli BRCA1 or BRCA2 mutation carriers were included in the training set (17 HGOC patients and 73 asymptomatic women), (BEDOCA trial; ClinicalTrials.gov Identifier: NCT03150121). The proteome of the microvesicle fraction of the samples was profiled by mass spectrometry and a classifier was developed using logistic regression. An independent cohort of 98 BRCA mutation carriers was used for validation. Safety information was collected for all women who opted for uterine lavage in a clinic setting. We present a 7-protein diagnostic signature, with AUC >0.97 and a negative predictive value (NPV) of 100% for detecting HGOC. The AUC of the biomarker in the independent validation set was >0.94 and the NPV >99%. The sampling procedure was clinically acceptable, with favorable pain scores and safety. Future research should focus on defining a classifier for an average-risk population, using the huge biorepository of proximal liquid biopsies.

S.12
Risk factors for ovarian cancer

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Based on the 2020 IARC's estimates, global ovarian cancer burden is projected to rise from 314 000 new cases in 2020 to 446 000 in 2040. Although ovarian cancer incidence is currently highest in high-income countries (various countries in Asia, Europe, North America), the predicted increases in the cancer burden will have the greatest impacts on low- and medium-income countries. Appropriate scaling up of resources for effective strategies in prevention is critical to effectively control the prevalence of adverse lifestyle factors and to ultimately reduce the cancer burden. However, primary prevention of ovarian cancer remains a challenge, given that the disease has relatively few known modifiable risk factors, particularly for the predominant, and lethal, high-grade serous subtype.

The aetiology of sporadic invasive ovarian cancer remains poorly understood. Relatively few classic lifestyle exposures are associated with risk of ovarian cancer. Studies in large consortia have shown substantial heterogeneity in the associations between well-established modifiable risk factors for ovarian cancer, such as smoking, use of oral contraceptives and use of menopausal hormone therapy, and disease risk by histotype. Emerging evidence suggests that inflammation-related exposures, including perineal use of talc-based body power, sexually transmitted infections (i.e., Chlamydia trachomatis), pelvic inflammatory disease, and use of anti-inflammatory analgesics may affect risk of ovarian cancer, and thus, are of increasing interest for prevention. However, additional epidemiologic and experimental data are required to confirm and clarify these associations. Prevention strategies for ovarian cancer in women at average risk remain elusive, given the limited number of known modifiable risk factors and the need to delineate target populations.

S.13
Risk-reducing surgery in hereditary and non-hereditary ovarian cancer – Con

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Risk reducing surgery has since long been suggested to carriers of BRCA1/2 gene mutations. The point of discussion here is the optimal time and the extent of surgery. The optimal time would be after childbearing has been completed, but is that after one, two, three or more children and at what age? The extent of surgery can be discussed. Salpingo-oophorectomy in pre-menopausal women will infer an increased risk of cardiovascular and osteoporotic disease, associated with a premature menopause. Salpingectomy after childbearing and a subsequent oophorectomy after menopause is an option considering the risks with premenopausal oophorectomy while the risk-reducing effect is more uncertain.

What is the risk-benefit balance with risk-reducing surgery in a low-risk population? If the lifetime risk for a woman to be diagnosed with epithelial ovarian cancer is less than 2%, how does that balance against the risk of performing surgery? Opportunistic salpingectomy has been suggested in conjunction with gynaecological surgery, particularly hysterectomy for a benign indication and laparoscopic tubal sterilization. Based on the theory that high-grade serous cancer is likely to develop in the Fallopian tube, the option to remove the site of origin before any development and spread of precancerous lesions from the tube to the ovary occurs, is appreciable. Does the additional procedure of bilateral salpingectomy carry an increased risk for surgical complications and impaired ovarian function and how are those risks valued by women?

Opportunistic salpingectomy has even been suggested in conjunction with any abdominal surgery, like cholecystectomy. Even less is known about the risks in this setting.

The above posed questions cannot be fully answered based on present knowledge. The most important message is that further research is needed to provide women with up-to date knowledge, to be able to make informed decisions about what organs they consent to being removed.

S.14
Risk-reducing surgery in ovarian cancer

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Ovarian cancer remains the most lethal of all gynecologic cancers and despite significant improvements in both surgical and medical treatments the past decade. Salpingectomy is associated with a lower risk for ovarian cancer development and may represent an important measure to prevent the disease. In this presentation, recent data and future perspectives related to opportunistic salpingectomy and ovarian cancer will be discussed.

S.15

Somatic Cancer Mutations in Pre-Malignant Gynecological Disease: lessons from molecular study of endometriosis

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Background: Clear cell and endometrioid ovarian cancers represent 20-40% of ovarian carcinomas and arise through malignant transformation of endometriosis. While cancer is thought to arise through an accumulation of genetics changes in normal cells and tissues, endometriosis is now recognised to harbour activating oncogene mutations without progressing to carcinoma.

Objectives: Examine features of somatic cancer-mutation harboring endometriosis to so-called wildtype disease. Examine the effects of KRAS oncogene activation in otherwise normal (murine) uterine epithelium and transplanted endometriosis model. Contrast our model to normal and cancer tissues.

Method: Mutation, protein, and RNA biomarkers were examined using sequencing, IHC, and NanoString platforms. Clinical data was collected through local and collaborator tissue banks and the Endometriosis Pelvic Pain Interdisciplinary Cohort Data Registry (EPPIC).

Results: Patients harboring somatic cancer driver mutations were more likely to be of higher stage and those affected by Deep infiltrating lesions, endometrioma, or multiple type of endometriosis were more likely to harbor mutations. Further, anatomically distinct lesions often carried clonal alterations suggesting endometriosis lesions, including those of distinct types, can share a common progenitor and may metastasize within an affected patient. Our model system highlighted Ras-pathway activation and abnormalities in gene expression cycling between pro-estrus and estrus.

Conclusions: Endometriosis represents a pre-malignant chronic neoplasm with many cancer-like properties including metastasis. Somatic alterations may lead to more persistent disease and, despite being insufficient for transformation, these alterations may contribute to overall risk. The study of endometriosis may highlight truncal, and targetable, features of endometriosis and their associated carcinomas. Treatment, diagnosis, and management of endometriosis, in its chronic state, has the potential to reduce future cancer burden.

S.16

Tumour and stroma responses to surgical trauma: coupling clinical, molecular and cellular data for improved ovarian cancer treatment

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Background: Contrary to expectation, we found that "state of the art" surgical treatment of women with advanced epithelial ovarian cancer, with significantly increased complete macroscopic resection rates, did not improve survival in our population-based and centralized health care setting.

Objectives: To examine the cellular and molecular effects of surgical trauma on the ovarian cancer cell and its microenvironments to serve as "proof of concept" to our clinical results by providing functional, transcriptomic and genomic insights that will be informative to understand the putative cellular and molecular alterations and mechanisms activated upon surgery.
Moreover, to lay the foundation to define prognostic biomarkers, to ultimately improve patient selection to surgical treatment and define new possible targets for treatment.

Method: In women subjected to upfront surgery with curative intent, patient samples (ascites, tumour, peritoneum and blood) are collected at the beginning and end of the surgical procedure. These samples are subjected to characterization of surgery-induced local molecular and cellular alterations in both the tissues and circulation in vivo, as well as ex vivo examination of the effects that these alterations have on the epithelial ovarian cancer cells aggressive functions. Systemic levels of inflammatory cytokines, growth factors and other biomarkers, circulating tumor DNA will be analyzed.

Results: The collection and processing of samples is completed. Intensive laboratory work is ongoing with 1) multiplexing of cytokine expression of serum, ascites, tumor tissue and peritoneum of the patient samples completed for half of the translational trial group (20/40 patients), 2) *ex vivo* functional assays for the impact of surgery-induced stromal inflammatory responses on cancer cell invasion, growth and chemoresistance have been conducted (40/40 patients) and the samples are currently being processed for detailed imaging and digital image analysis, 3) Immune cell profiling using flow cytometry and immunofluorescence is underway. Results are expected during 2024.

Conclusions: Awaiting results.

S.17
Understanding the non-genetic mechanisms of therapy resistance

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Background: Resistance to available therapies still limits overall survival in cancer. Therapy resistance is the outcome of genetic and non-genetic changes in cancer cells during treatment. Although genetic alterations are well-documented, little is known about non-genetic modifications. Critically, we lack tools that could inform us whether a patient will respond to a therapy or display resistance. Therefore, we need to explore non-genetic mechanisms of therapy resistance more in-depth and take initiatives to develop therapy response prediction tools.

Objective: This study aims to develop drug sensitivity prediction tools and apply the knowledge to study the mechanisms of adaptive non-genetic mechanisms of therapy resistance.

Methods: We used pharmacogenomic data to develop machine learning algorithms for drug sensitivity prediction. Multi-omics approaches were applied to map the elements regulating drug sensitivity. Biochemical assays were used for target verification and exploring cellular signaling in cells and animal models.

Results: We developed venetoclax and cisplatin sensitivity prediction tools using deep learning algorithms. We observed that venetoclax sensitivity is regulated by the polo-like kinase 1 (PLK1) activity and can be improved by PLK1 inhibition. Mechanistically PLK1 transcriptionally regulates the pro-apoptotic protein NOXA. Expression of NOXA results in inhibition of the anti-apoptotic protein BCL2 that in turn enhances venetoclax sensitivity.

Conclusion: Data suggest a novel mechanism of how venetoclax sensitivity is regulated and identifies a link between PLK1 and BCL2 family proteins that contributes to therapy resistance.

S.18
What is optimal Ovarian Cancer Surgery - When, How and to Whom?

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Background: Ovarian cancer has the highest mortality rate of all gynecological cancers. The majority is diagnosed at advanced stages and recurrences are common. Primary treatment consists of surgery, in particular radical extensive surgery, combined with systemic chemotherapy and targeted therapies. Extensive surgery aiming for complete cytoreduction to no residual disease is considered important for optimizing survival. However, there has been a debate during the last years concerning how, when and to whom the extensive surgery should be performed and the efficacy of surgery for recurrences.

Objectives: To present an update on the international scientific research behind the surgical recommendations in different aspects in ovarian cancer.

Method: An overview of the literature concerning primary debulking surgery, interval debulking surgery after neoadjuvant chemotherapy, hyperthermic intraperitoneal chemotherapy (HIPEC) and surgery for recurrent disease in ovarian cancer.

Results: Randomized controlled trials are scarse, but there are observational and population-based studies indicating that primary debulking surgery with complete cytoreduction is associated with the highest survival rate followed by interval debulking. HIPEC after neoadjuvant chemotherapy at the interval debulking surgical procedure has been shown promising although needs to be confirmed. Recurrent surgery has been proven to have an impact on survival when complete cytoreduction can be achieved. Patient selection for all the different surgical methods is pivotal and multidisciplinary discussions important.

Conclusions: Surgery for ovarian cancer is one of the cornerstones in the primary treatment and patient selection is crucial although still not fully clear to whom and how to perform the surgical procedure for best outcome and survival.