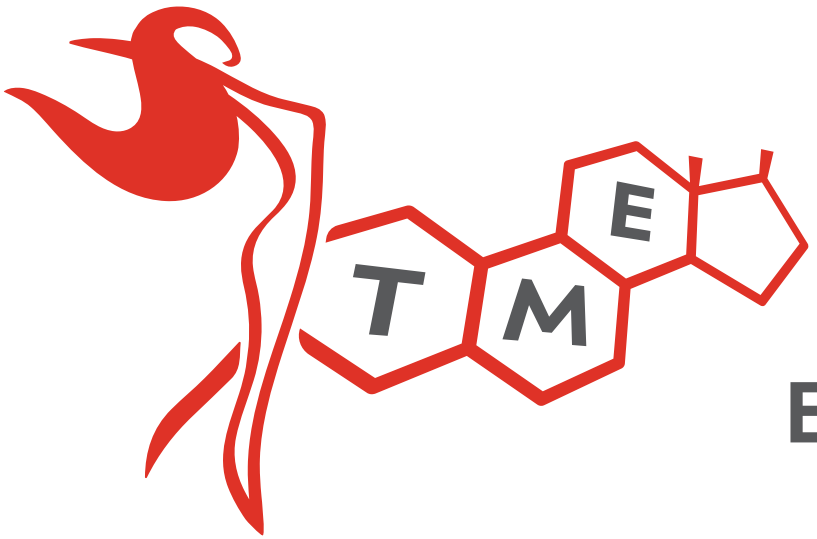

TRANSLATIONAL MOLECULAR ENDOCRINOLOGY

BOOK OF ABSTRACTS 2025

Organized by the Program group P3-0449 of the Faculty of Medicine, University of Ljubljana and the Association of Gynecologists and Obstetricians of Slovenia.

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2. Scientific meeting Translational Molecular Endocrinology

17th September 2025

Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

BOOK OF ABSTRACTS

2025

Dear Colleagues,

In 2024, we organized the first scientific meeting of the newly founded *Translational Molecular Endocrinology for Women's Health* programme group with invited speakers from abroad. The meeting was very well received, so we decided to make this event a tradition and organize it annually. With this meeting we would like to provide a platform for Slovenian and collaborating groups, from EU and non-EU countries, to present and discuss their current studies in the field of translational molecular endocrinology, as well as give patients the opportunity to present their views on research in the field of hormone-dependent diseases. All in all, this meeting should help to broaden the horizons of all participants.

The 2nd scientific meeting "**Translational Molecular Endocrinology**" comprises four sessions. In the first session, **models for hormone-dependent diseases**, various *in vitro* models, from primary cell cultures, to 3D and multicellular organoid models, organ-on-chip platforms and patient-derived explants, are presented and discussed in the context of ovarian cancer, in particular adaptations of serine metabolism in chemoresistant HGSOC, the effects of semaglutide on endometrial maturation and embryo implantation, and infertility.

The second session focuses on the **molecular mechanisms of hormone-dependent diseases**, specifically modelling androgen metabolism in HGSOC, pathways associated with increased BMI in infertile women, and WNT signaling in endometrial cancer. Presentations on new options for the treatment of hormone-dependent diseases include an update on HSD17B1 inhibitors using docking and molecular dynamics simulations, and MPA and mefenamic acid repurposing for the treatment of HGSOC.

Diagnostic and prognostic biomarkers for hormone-dependent diseases are the topic of the third session. This session includes two studies on endometriosis: a study in menstrual discharge supporting non-invasive diagnostics and a proteomics analysis in uterine lavage identifying proteins associated with infertility, and two studies on cancer: a study on the ATP7A transporter as a potential biomarker for chemoresistant HGSOC and a multisteroid profiling identifying androgens as promising biomarkers for endometrial cancer.

The fourth session provides **patients' perspective in research into hormone-dependent diseases:** with presentations from representatives of endometriosis patients (Endozavest Association) and patients with gynecological cancer.

The meeting provide ample time for networking and establishing new contacts and collaborations that will contribute to high-quality studies in the field of translational molecular endocrinology in the coming years.

We would like to thank the invited speakers, all presenters, the members of the programme group, and everyone involved in the organization of this event.

We cordially invite you to the 3rd Translational Molecular Endocrinology Meeting in September 2026**.**

On behalf of the organizing committee

Head of the program group P3-0449

Prof. dr. Tea Lanišnik Rižner

Scientific Meeting Translational Molecular Endocrinology*

17th September 2025

University of Ljubljana, Faculty of Medicine, Korytkova 2, 1000 Ljubljana, Slovenia

10:00-10:05	**Welcome**
	Vita Dolžan, Head of the Institute of Biochemistry and Molecular Genetics, UL MF, Ljubljana, Slovenia **Borut Kobal**, Head of the Division of Gynaecology and Obstetrics, UMC Ljubljana, Slovenia
10:10-11:20	**Models of Hormone Dependent Diseases**
	Chairs: Daniela Annibali and Maja Pušić Novak
10:10-10:30	**Daniela Annibali**, KU Leuven, Leuven, Belgium *Deciphering Serine Metabolism Adaptations in Platinum Resistant Ovarian Cancers: Concepts and Tools*
10:30-10:50	**Gaby Steba**, UMC Utrecht, Netherlands *Using 3D Endometrial Organoid Models to Study Infertility*
10:50-11:05	**Amruta Pathare**, CELVIA, Tartu, Estonia *Semaglutide and Embryo Implantation: Insights from Hormonally Treated In-Vitro Models*
11:05-11:20	**Maja Pušić Novak**, UL MF, Ljubljana, Slovenia *Phenotypic and Functional Characterization of Primary Endometriotic Stromal Cells*
11:20-12:00	**Coffee break and Poster session**
12:00-13:30	**Molecular Mechanisms of Hormone Dependent Diseases**
	Chairs: Karl Storbeck and Marija Gjorgoska
12:00-12:20	**Karl Storbeck**, Stellenbosch University, Stellenbosch, South Africa *Computational Modeling of Androgen Metabolism in High-Grade Serous Ovarian Cancer*
12:20-12:35	**Vesna Šalamun**, University Medical Centre Ljubljana, Slovenia *Show Me Your Endometrial Transcriptome and I Will Tell You Your Body Mass*
12:35-12:50	**Živa Ledinek**, University Medical Centre Maribor, Slovenia *The Influence of WNT Signalization and EMT on Endometrial Cancer Characteristics*
12:50-13:10	**Jurica Novak**, Rudjer Bošković Institute, Zagreb, Croatia *Update on Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1: Docking and Molecular Dynamic Simulations*
13:10-13:25	**Nika Marolt**, UL MF, Ljubljana Slovenia *Repurposing MPA and Mefenamic Acid for HGSOC: Inhibition of AKR1C as a Therapeutic Strategy*
13:25- 15:00	**Lunch break and Poster session**

15:00-16:10	**Diagnosis and Prognosis of Hormone Dependent Diseases**
	Chairs: Polona Šafarič Tepeš and Jerzy Adamski
15:00 - 15:20	**Polona Šafarič Tepeš**, Northwell Health, New York, USA *Natural Biopsies of the Endometrium: Insights from Menstrual Effluent into Iron-Regulated Migration pathways in Endometriosis*
15:20-15:35	**David Lukanović**,University Medical Centre Ljubljana, Slovenia *Can ATP7A Transporter Serve as a Biomarker for Predicting Chemoresistance in High Grade serous Ovarian Cancer?*
15:35-15:50	**Marija Gjorgoska**, UL MF, Ljubljana, Slovenia *Multi-steroid Profiling and Machine Learning Reveal Androgens as Promising Biomarker Candidates for Endometrial Cancer Diagnosis*
15:50-16:05	**Edi Muhaxhiri**, University Medical Centre, Ljubljana, Slovenia *Proteomic Profile of Uterine Cavity Lavage in Endometriosis Patients - Promising Approach for Biomarker Discovery and Pathophysiology Explanation*
16:10- 16:30	**Research on Hormone Dependent Diseases – Patients' Perspectives**
	Chair: Vid Janša
16:10-16:20	**Amber Bervar**, Endozavest, Slovenia
16:20-16:30	**Polona Selič-Zupančič**, Slovenia
16:30-17:00	**Concluding Remarks**
	Tea Lanišnik Rižner, program group Translational Molecular Endocrinology for Women's Health, UL MF, Ljubljana, Slovenia
17:30 -18:30	**Social event: Ljubljanica boat tour**

*Organized by the Laboratory for Translational Molecular Endocrinology UL MF and

Association of Gynecologists and Obstetricians of Slovenia.

ZDRUŽENJE GINEKOLOGOV IN PORODNIČARJEV SLOVENIJE

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Models of Hormone Dependent Diseases

Deciphering Serine Metabolism Adaptations in Platinum Resistant Ovarian Cancers: Concepts and Tools

Daniela Annibali[1], Tom van Nyen[1], Mélanie Planque[2], Alejandro herreros-Pomares[1], Giovanni Esposito[1], Hugo M. Horlings[3], Ben Davidson[4], Sarah-Maria Fendt[2], Frédéric Amant[1,5,6]

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Resistance to platinum-based chemotherapy represents a major clinical and societal challenge for the management of many cancer patients, particularly those with epithelial ovarian cancer, for whom median survival does not exceed 12-15 months. Critically, our understanding of lethal disease remains limited because routine sampling of late-stage tumors and affected tissues is rarely performed, resulting in a major knowledge gap. At the same time, it is becoming evident that development of resistance goes beyond specific genetic mutations. We recently discovered a striking example of ovarian cancer plasticity potential in response to therapy. Serine biosynthesis has been linked to cancer growth and poor prognosis in various cancer types, but its role in platinum-resistant ovarian cancer was not investigated before. By using metabolomics approaches and patient-derived preclinical models, we found that a subset of ovarian cancers, upon platinum exposure, evades lethality by downregulating serine biosynthesis pathways, thus becoming dependent on exogenous serine to sustain PARP activity and DNA repair. Remarkably, this flexibility can partially resensitize them to platinum. Our findings underscore the critical role of microenvironment-driven mechanisms and plasticity in resistance and the need of relevant models to functionally validate novel potential targets.

Funding:
FWO (Research Fund Flanders)
KOTK (Standup Against Cancer)

Using 3D Endometrial Organoid Models to Study Infertility

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Historically, infertility research has predominantly focused on the embryo. However, recent advances have shifted attention toward the endometrium and its dynamic interaction with the embryo. A major breakthrough in this field has been the development of 3D endometrial organoids, which offer a physiologically relevant model of the human endometrium. Building on this foundation, we are now developing more complex systems, including multicellular organoid models, organ-on-a-chip platforms, and integrated multi-organ-on-a-chip technologies, often incorporating microfluidic techniques. These innovations open new avenues for investigating previously inaccessible aspects of human reproductive biology. Yet, a critical question remains: how complex do these models need to be to effectively address specific research questions? This presentation will explore the balance between model complexity and research utility in the context of infertility studies. Furthermore, some of the latest developments from our lab will be discussed, showcasing new directions and applications in this rapidly evolving field.

Funding: GS Steba was supported by department funds

Semaglutide and Embryo Implantation: Insights from Hormonally Treated In-vitro Models

Amruta D. S. Pathare[1,2,3], Apostol Apostolov[1,2,3,4], Darja Lavõgina[1,5,6], Keiu Kask[1,7], Sanjiv Risal[2,3], Susana Ruiz-Durán[8,9], Aive Kalinina[10], Triin Tammiste[7,11], Merli Saare[1,7], Maire Peters[1,7], Ganesh Acharya[2,12,13], Fredrik Lanner[2,3,14], Alberto Sola-Leyva[1,2,3], Andres Salumets[1,2,3,7]

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Glucagon-like peptide-1 receptor (GLP-1R) was found to be expressed in the human endometrium across the menstrual cycle, with the highest levels during the mid-secretory phase. Semaglutide, a GLP-1 receptor agonist (GLP-1RA), induced dynamic and dose-dependent transcriptomic changes in endometrial epithelial organoids (EEOs), primary stromal cells (ESCs), and blastoids. While GLP-1RA therapy has been proven effective in glycaemic control and is an emerging treatment for type 2 diabetes, obesity, and polycystic ovarian syndrome (PCOS), its impact on endometrial receptivity and embryo development remains poorly understood. To address this, we investigated semaglutide effects in paired ESCs and EEOs in vitro models derived from fertile women, stimulated with oestradiol, progesterone, and cyclic adenosine monophosphate to mimic receptive and decidualized states, as well as in blastoids derived from H9 cells. GLP-1R expression was assessed by immunohistochemistry, cell viability by resazurin assay, cAMP levels by biosensor assay, decidualization by prolactin secretion, and transcriptomic changes by RNA sequencing.
Semaglutide significantly and dose-dependently reduced ESC metabolic activity, with reduced viability observed in EEOs only at the highest concentration (5 µM). Both ESCs and EEOs showed increased cAMP production in response to semaglutide, and EEOs upregulated

receptivity-associated genes including PAEP, LIF, and SPP1. Transcriptomic analysis revealed that 39 nM semaglutide did not alter gene expression, whereas 1.25 µM induced 10 differentially expressed genes (DEGs). At 5 µM, semaglutide profoundly altered endometrial cell transcriptomes: EEOs exhibited more than 400 DEGs linked to metabolic processes, mitochondrial function, and receptivity, while ESCs showed downregulation of cell cycle and proliferation genes. In blastoids, semaglutide promoted expression of pluripotency and endoderm differentiation genes (EPAS1, PBX1, TDGF1, PRDM14, SOX2) while suppressing metabolic pathways. This study reveals fundamental mechanistic effects of semaglutide on endometrial maturation and embryo development, highlighting potential implications for fertility treatment. Further evaluation in obese and PCOS women is required to establish translational relevance and clinical safety.

Funding: This study was supported by the Estonian Research Council (grants nos. PRG1076 and PSG1082), the Swedish Research Council (grant no. 2024-02530), and the Novo Nordisk Fonden (grant no. NNF24OC0092384).

Phenotypic and Functional Characterization of Primary Endometriotic Stromal Cells

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Endometriosis is a chronic gynecological disorder affecting approximately 11% of women in their reproductive age. Although primary endometriotic cell cultures are already valuable research models of endometriosis, they often lack thorough characterization in terms of purity and phenotypic profiling. The aim of this study is to establish and characterize primary endometriotic stromal cell cultures that could be used as relevant *in vitro* models and for development of a novel immortalized endometriotic cell line.

Tissue samples (n=18) were collected from patients with endometrioma (n=3), peritoneal lesions (n=14), and rectovaginal septum (n=1) under a standardized protocol at the Department of Gynaecology, UMC Ljubljana. From the collected samples, primary stromal cells were isolated and screened for mycoplasma. They were further evaluated for viability, doubling time, migration capacity, expression of estrogen- related genes, capacity to form spheroids, response to hormones (estradiol, E2, medroxyprogesteroneactetate, MPA), and immunocytochemical expression of vimentin, cytokeratin, and Pax2.

After isolation of cells, four cultures failed to proliferate and couldn't be used for further analysis. The remaining cells maintained >90% viability through passage 5, with a mean doubling time of 2.33 ± 0.94 days. All tested negative for mycoplasma. Wound healing assays showed closure within an average of 62 h. All primary cultures exhibited expression of *ESR1, ESR2*, *GPER versions 2, 3, 4, PGR, HSD17B1, HSD17B2, IL1β* and *STAR* genes and proteins vimentin (stromal marker) and Pax2 (endometrial marker). All primary cultures were able to form compact spheroids within 24 hours after seeding. Response of primary cultures to the treatment with MPA and E2 varied between patients.

We successfully established well-characterized primary endometriotic stromal cell cultures with high viability, migration capacity and defined phenotype. These primary cultures provide a reliable platform for modeling endometriosis and serve as a foundation for generating a novel immortalized cell line.

Funding: This research was supported by ARIS grants Z3-4522 to M.P.N. and P3-0499 to T.L.R, and the EU H2020-MSCA-RISE project TRENDO.

3D Bioprinted Ovarian Cancer Models: a Physiologically Relevant Platform for Evaluating Chemotherapy Response and Tumor Biology

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Ovarian cancer (OC), notably high-grade serous ovarian carcinoma (HGSOC), is characterized by significant heterogeneity and therapeutic resistance. In 2022, OC was the 10th most frequent cancer among women globally, affecting 324,603 individuals with an age-standardized incidence rate (ASR) of approximately 6.0 per 100,000 women. It caused 206,956 deaths worldwide, representing an ASR of 4.0 per 100,000 women, ranking among the leading causes of cancer-related mortality. Despite initial treatment success using surgical intervention and platinum-based chemotherapy, over 80% of patients subsequently develop chemoresistance, drastically limiting long-term survival, with 5-year rates at only 40% for stage III and 20% for stage IV disease.

Recent advances highlight the importance of molecular characterization, identifying four distinct molecular subtypes of HGSOC—proliferative, immunoreactive, differentiated, and mesenchymal—each impacting treatment response and prognosis. To address these clinical challenges, innovative 3D bioprinted tumor models have emerged as superior *in vitro* platforms. Our work employed extrusion-based 3D bioprinting technology BIO X™ bioprinter using OVSAHO cell lines encapsulated in Laminik+ bioink, creating lattice structures mimicking ovarian tumor microenvironments. These models demonstrated consistent architectural fidelity, viability, and critical cell-cell interactions essential for realistic tumor modeling. Such advancements in bioprinting provide a scalable, physiologically relevant alternative for drug screening, personalized medicine, and elucidation of ovarian cancer biology.

Viability assays using LIVE/DEAD™ staining demonstrated sustained peripheral cell viability and central necrosis indicative of nutrient and oxygen diffusion gradients. Histological analysis confirmed dense cellular architecture and ECM deposition consistent with tumor tissue.
To assess the functional applicability, we evaluated carboplatin-induced cytotoxicity. Constructs treated with increasing carboplatin concentrations exhibited dose-dependent cytotoxic responses. The IC50 was determined using the ToxiLight BioAssay Kit, demonstrating the bioprinted model's capability to evaluate chemotherapy responses.

Funding: ARIS project L4-4565 and BI-ME/25-27-013 (I.J.) and programme grants P3-0449 (T.L.R.), P3-0108 (M.E.K.), P1-0245 and P1-0390; European Union HE CutCancer project (101079113).

Gene Expression Signatures of Carboplatin Resistance in High-Grade Serous Ovarian Cancer: Comparative Insights from 2D and 3D Models

Vesna Kokondoska Grgič[1,2], Nika Marolt[1], Ajla Kubat[1], Tamara Dokmanović[1], Katja Kološa[3], Ivana Jovčevska[1], Tea Lanišnik Rižner[1]

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High-grade serous ovarian cancer (HGSOC) is a major medical problem because it often does not respond well to platinum-based chemotherapy. This study investigated gene expression profiles associated with carboplatin sensitivity using 2D and 3D ovarian cancer cell culture models (OVSAHO, OVCAR4, Kuramochi, COV362). Utilizing publicly available transcriptomic data, we identified key differentially expressed genes involved in critical pathways such as DNA repair, epithelial-mesenchymal transition (EMT), matrix remodeling, hypoxia, and angiogenesis. We found important links between gene expression levels and how resistant the cancer cells are to carboplatin, pointing out MMP2, MMP9, and genes related to EMT (SNAI1, ZEB1, VIM) as possible indicators of this resistance.

Kaplan-Meier survival analyses showed that MMP9 and TWIST2 are important for predicting patient outcomes based on gene expression. Quantitative PCR (qPCR) validation *in vitro* confirmed differential expression patterns between sensitive and resistant models. Additionally, we showed that 3D ovarian cancer spheroids were much more resistant to carboplatin than the 2D versions, which closely reflects what happens in clinic treating real patients.
Flow cytometry analysis showed that treating with IC50 carboplatin successfully stopped the cell cycle and slowed down cell growth, suggesting that the cells were not dying but rather just not dividing. This effect was consistently observed across cell lines, underlining the model's clinical relevance.

This research emphasizes the value of integrating advanced 3D models to better capture HGSOC biology and therapeutic responses, ultimately aiming to improve prognostic accuracy and inform targeted treatment strategies.

Funding: ARIS project L4-4565 and BI-ME/25-27-013 (I.J.) and programme grants P3-0449 (T.L.R.); European Union HE CutCancer project (101079113).

3D Spheroids From Primary Endometriotic Stromal Cells Reveal Variable Responses to Estradiol and Medroxyprogesterone Acetate

Maruša Godler[1], Helena Ban Frangež[2], Tea Lanišnik Rižner[1], Maja Pušić Novak[1]

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Endometriosis is a chronic, estrogen-dependent gynecological disease in which endometrial-like tissue grows outside the uterus, causing pelvic pain and infertility. To better replicate its complex environment, advanced *in vitro* models such as 3D spheroids have been developed for studying hormonal responses and testing new therapies. This study aimed to establish 3D spheroids from primary endometriotic stromal cells and evaluate the effects of estradiol (E2) and medroxyprogesterone acetate (MPA) on spheroid growth, alongside a parallel 2D migration assay.

Endometriotic tissue samples from patients with peritoneal endometriosis (PE) (n=9) were collected at the Department of Gynaecology, UMC Ljubljana. Primary stromal cells were isolated, screened for mycoplasma, and cells from five patients were used to optimize seeding density. On day 5, spheroids were treated with 10 µM E2, 10 µM MPA, or DMSO control and monitored for 5 days. Parallel 2D cultures underwent scratch assays with identical treatments applied immediately post-scratch. Imaging was performed every 4 h using an Incucyte S3 (Sartorius, Germany). HIESC (stromal cells of normal endometrium) served as control, while Z12 (epithelial endometriotic cells of PE) were included to assess response of epithelial cells to the treatments.

Spheroids formed within 24 h, compacted and reached stable size after day 5. Roundness varied between patient-derived cultures. In control cell lines, Z12 and HIESC, E2 increased while MPA decreased spheroid size. In primary stromal spheroids, E2-driven growth was seen in two patients while in others it showed no change. MPA effects on spheroid size varied between patients. In migration assays, Z12 and HIESC closed wounds faster with both treatments compared to controls, whereas primary cultures showed heterogeneous responses: E2 accelerated closure in some, while MPA slowed it in most.

These results agree partly with previous reports of E2 promoting proliferation and motility, and progestins showing variable effects, possibly due to progesterone resistance. Inter-patient variability underscores the heterogeneity of endometriosis and supports personalized *in vitro* models for therapy testing.

Funding: This research was supported by ARIS grants Z3-4522 to M.P.N. and P3-0499 to T.L.R, and the EU H2020-MSCA-RISE project TRENDO.

Molecular Mechanisms of Hormone Dependent Diseases

Computational Modeling of Androgen Metabolism in High-Grade Serous Ovarian Cancer

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Hormone-dependent cancers are driven by sex steroids (androgens and estrogens) and rely on both peripheral and local hormone activation. Recently, a novel group of adrenal-derived androgens, known as the 11-oxygenated androgens, has been identified in humans. The active forms, 11-ketotestosterone (11KT) and 11-keto-5α-dihydrotestosterone (11KDHT), are potent androgen receptor (AR) agonists with activities comparable to testosterone and DHT, respectively. Notably, 11KT is the most abundant circulating active androgen in postmenopausal women and in men with castration-resistant prostate cancer (CRPC). While 11-oxygenated androgens have been implicated as drivers of CRPC, their contribution to hormone-dependent cancers in women, such as ovarian cancer, has not yet been determined.

Given the challenges in obtaining tissue samples and the difficulty of measuring intratumoural steroid levels, we are employing a bottom-up systems biology approach to develop a comprehensive computational model of classic and 11-oxygenated androgen metabolism that can be used as a predictive tool. Our aim is to develop a model that can be tailored to the enzyme expression profiles and circulating steroid levels of a specific cancer, enabling predictions of intratumoural steroid concentrations and the impact of altered enzyme expression or inhibition. Here, we present a proof of concept by demonstrating the model's ability to predict both classic and 11-oxygenated androgen metabolism in six high-grade serous ovarian cancer (HGSOC) cell models.

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Show Me Your Endometrial Transcriptome and I Will Tell You Your Body Mass

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Obesity significantly affects female fertility, primarily through impaired ovulation and endometrial receptivity. However, the specific transcriptional changes in the endometrium linked to elevated body mass index (BMI) remain poorly understood.

We analyzed endometrial transcriptomic profiles in infertile obese women (BMI >30 kg/m²) compared to women with normal BMI. Biopsies were collected during the mid-luteal phase (days 21–23 of the menstrual cycle).

RNA sequencing and differential gene expression analysis revealed 4,968 genes significantly correlated with BMI ($p<0.05$). After correction for multiple testing, 113 genes remained differentially expressed. Notably, GSK3B, IDO1, ALDH1A3, and PGR showed the strongest associations, with GSK3B expression particularly correlated with body weight.

Gene ontology and pathway enrichment analyses identified several biologically relevant pathways linked to increased BMI, including inflammatory signaling, thyroid hormone signaling, Wnt signaling, glucocorticoid receptor activity, and amino sugar metabolism. A key finding was activation of the TGF-β pathway, supporting a role for low-grade chronic inflammation in obesity-related endometrial dysfunction.

These findings suggest that the endometrial transcriptome reflects body mass and may serve as a molecular fingerprint of metabolic influences on endometrial function. This may explain how obesity impairs implantation and pregnancy outcomes, even when oocyte quality is preserved.

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EMT and Wnt Markers Correlate with Hormone Receptor Status in EC

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Two of most important and partially intertwined molecular pathways, linked to the carcinogenesis and progression of endometrial cancer (EC), are Wnt signalling and epithelial-to-mesenchymal transition (EMT). Wnt signalling leads to nuclear accumulation of β-catenin and transcription of cell-cycle regulator genes and is regulated by various Wnt inhibitors, such as Dkk proteins. EMT is a process, characterized by loss of epithelial (E-cadherin) and gain of mesenchymal (N-cadherin) markers and has been associated with the process of metastasis. Better understanding of mechanisms, leading to EC progression is important for adequate treatment planning and can influence the prognosis of the patient.

We aimed to compare the expression of markers, associated with Wnt signalling (β-catenin and Dkk1) as well as EMT (E-cadherin and N-cadherin) with hormone receptor status in women who underwent hysterectomy for the treatment of EC. In addition to standard histopathological report, molecular classification of tumours was done by determining POLE mutation status as well as p53 and MMRd expression, which was done by immunohistochemistry (IHC). To assess the influence of Wnt signalling ad EMT, β-catenin, Dkk1, N-cadherin and E-cadherin expression was evaluated, using IHC. We compared the expression of beforementioned tumour characteristics with hormone receptor status, using non-parametric statistical tests.

Expression of oestrogen receptors was found to be positively correlated with expression of β-catenin, N-cadherin and Dkk1, expression of progesterone receptors was positively correlated with expression of β-catenin and expression of androgen receptors was positively correlated with expression of β-catenin and N-cadherin. Tumours with at least focal nuclear expression of β-catenin had higher expression of all three hormone receptors.

Our results show that hormone status correlates with expression of Wnt signalling and EMT markers, showing that signalling pathways leading to development and progression of EC might be hormone dependent. Further studies are needed to validate our results on larger cohort of patients.

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Update on Inhibitors of 17β-hydroxysteroid Dehydrogenase Type 1: Docking and Molecular Dynamic Simulations

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Researchers explored how five small molecules (01D, 05A, 06A, 11D, and 12M) interact with the human enzyme 17β-hydroxysteroid dehydrogenase type 1, which plays a key role in hormone regulation. Using advanced computer simulations, they examined how tightly each molecule binds to the enzyme and how stable these connections remain over time.

The work began with docking — a method that predicts how each molecule fits into the enzyme's active site. Then, long molecular dynamics simulations were run in triplicate to mimic realistic movement and flexibility in a cell-like environment.

Results showed that 05A and 11D formed the most stable complexes, while 06A and 12M caused more structural movement in a flexible tail region. Energetic calculations ranked 05A and 06A as the strongest binders, with 12M performing the worst, sometimes even drifting out of the active site.

Analysis of contact patterns revealed that hydrophobic (water-repelling) forces dominated binding, with a small set of key amino acids — especially Leu150, Pro188, Phe260, and Tyr156 — playing a major role in holding the inhibitors in place.

Overall, 05A and 06A appear the most promising for future drug design, combining strong fit, stable binding, and favorable chemistry.

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Repurposing MPA and Mefenamic Acid for HGSOC: Inhibition of AKR1C as a Therapeutic Strategy

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High-grade serous ovarian carcinoma (HGSOC) is the most prevalent and lethal subtype of ovarian cancer, frequently characterized by resistance to platinum-based chemotherapy. Members of the aldo-keto reductase subfamily 1C (AKR1C) have been associated with both chemoresistance and hormone-regulated tumor progression, though their exact contribution to HGSOC remains to be fully elucidated. To investigate this, we analyzed transcriptomic profiles from The Cancer Genome Atlas (TCGA) to assess expression patterns of *AKR1C1–3* and *NFE2L2* (encodes NRF2, a key oxidative stress regulator) in HGSOC tumors. Notably, platinum-resistant samples exhibited stronger co-expression relationships among *AKR1C* genes. Kaplan–Meier Plotter analysis revealed that elevated expression of *AKR1C1*, *AKR1C2*, and reduced expression of *NFE2L2* was associated with reduced survival in patients with serous ovarian cancer. qPCR and RNAseq analysis confirmed higher expression of *AKR1C1–3* in the least carboplatin-sensitive cell lines (Caov-3 and COV362), although *NFE2L2* expression patterns did not consistently reflect those seen in clinical survival data. We next evaluated the antitumor activity of two FDA-approved AKR1C inhibitors, medroxyprogesterone acetate (MPA) and mefenamic acid (MEF). Both agents significantly decreased cell viability and impaired migratory capacity, whether administered alone or in combination with estrone sulfate and/or carboplatin. In several assays, their efficacy matched or exceeded that of carboplatin. Importantly, MPA and MEF induced apoptosis without triggering necrosis in either cell line. In contrast, carboplatin failed to activate apoptotic pathways and caused delayed necrosis only in Caov-3 cells after 72 hours, suggesting a slow, non-apoptotic mechanism of cell death. Together, these results highlight *AKR1C* and *NFE2L2* as candidate prognostic markers in HGSOC and suggest that repurposing MPA and MEF may offer a promising therapeutic strategy against platinum-resistant tumors.

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Cellular and Molecular Responses to Simulated CO_2 Pneumoperitoneum in Cervical and Endometrial Cancer Models

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Carbon dioxide (CO_2) insufflation to create a pneumoperitoneum is standard practice in minimally invasive surgery (MIS). While MIS is used in gynecologic oncology, including for hormone-dependent cancers, its application in cervical cancer has raised concerns due to associations with poorer oncologic outcomes. In contrast, such outcomes have not been observed in endometrial cancer. The biological basis for this discrepancy remains unclear, though some studies suggest that CO_2 insufflation may contribute to it. To explore this possibility, we investigated the effects of CO_2 insufflation on gynecologic cancer cell behavior at both the cellular and molecular levels.

To simulate surgical conditions, we established *in vitro* models replicating both CO_2 pneumoperitoneum at 15 mmHg insufflation and ambient conditions representative of open surgery. Six gynecologic cell lines were used: three cervical (C-33A, HeLa, CaSki), representing diverse HPV statuses and histologic subtypes; and three endometrial (HIEEC, Ishikawa, KLE), ranging from non-neoplastic to carcinomatous. Cellular responses—including proliferation, viability, and migration—were assessed up to three days post-exposure using the Alamar Blue assay, Calcein-AM/Propidium Iodide (Calcein/PI) staining, and the scratch wound-healing assay. Media pH was also monitored. Transcriptomic changes were analyzed via RNA sequencing.

CO_2 exposure resulted in increased migration in specific cell lines, suggesting a potential for enhanced invasiveness under insufflation conditions. Overall, cell survival was largely unaffected, with only minor changes in viability observed. Transcriptomic analysis revealed that CO_2 insufflation induced distinct alterations in gene expression profiles. Our findings suggest that CO_2 pneumoperitoneum may modulate tumor cell behavior by promoting motility in certain gynecologic cell lines. Due to the heterogeneity of tumor responses, further studies are warranted to determine the clinical relevance of these *in vitro* observations in the context of surgical oncology.

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Assessment of Hormonal Receptors' Expression in Fine-needle Aspiration Biopsy Samples of Breast Adenocarcinoma and Correlation with Histology

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Assessment of estrogen (ER) and progesterone (PR) receptors is essential for selecting patients for targeted hormonal therapy in breast cancer. Receptor status can be determined on both cytological and histological samples, with cytology offering faster results. To ensure reliability, cytology must be continuously validated against histology. This study investigated the concordance of ER and PR expression in paired cytological and histological samples from patients treated at the Institute of Oncology Ljubljana. A retrospective analysis of 98 samples (94 breast tumors, 4 lymph nodes) collected between 2023 and early 2025 was performed. Immunocytochemical and immunohistochemical results were retrieved from the laboratory information system for patients who underwent fine-needle aspiration biopsy, core needle biopsy, or surgical biopsy. Median ER expression was 70% (range 0–100%) in cytology and 100% (range 5–100%) in histology, while PR showed a median of 5% (range 0–90%) in both cytology and histology, with broader distributions in histology. Expression levels of both ER and PR were generally lower in cytology ($p<0.001$) but strongly correlated with histology (ER: $\rho=0.78$, $p<0.001$; PR: $\rho=0.84$, $p<0.001$), meaning that cases with high expression levels in histology also had higher levels in cytology, and those with low histology levels were lower in cytology. Reliability was very good for ER ($\alpha=0.84$) and PR ($\alpha=0.70$). Our analysis confirms the reliability of cytology for accurate hormone receptor evaluation and supports its use in routine breast cancer diagnostics for ER and PR assessment.

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Effect of Selected Drugs on the Activity of the Enzyme AKR1C3

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Aldo-keto reductase 1C3 (AKR1C3) catalyses the reduction of the carbonyl to the hydroxyl group in the biosynthesis of androgens and estrogens. AKR1C3 is involved in the development and progression of breast and prostate cancer and represents novel drug target. We aimed to test two hypothesis. First, AKR1C3 is present in various hormone-dependent cancer cell lines. Second, the drugs already used in clinical practice: nonsteroidal anti-inflammatory drugs (flufenamic, mefenamic, meclofenamic acid) and hormonal drugs (medroxyprogesteron acetate, ulipristal acetate, dienogest) decrease the activity of recombinant enzyme AKR1C3. The presence of AKR1C3 in cancer cell lines was assesed by Western blot using specific and validated antibodies. The activity of AKR1C3 enzyme was measured spectrophotometrically by monitoring the oxidation of substrate 1-acenaphthenol to 1-acenaphthenone, with simultaneus reduction of $NADP^+$ to NADPH. We measured the change in absorbance at 340 nm with time and determined the initial reaction rates. By comparing the initial rates of reaction in the presence of increasing concentrations of selected drugs, the IC50 values were determined. AKR1C3 protein was detected in most breast, endometrial, and ovarian cancer cell lines. The selected drugs inhibited the activity of the AKR1C3 enzyme (Student t-test, $p < 0.05$). Medroxyprogesteron acetate was the most effective inhibitor with an IC50 value of 0.06 µM, followed by meclofenamic acid, mefenamic acid, flufenamic acid and ulipristal acetate, with IC50 values of 1.8 µM, 2.0 µM, 3.7 µM and 5.1 µM, respectively. Dienogest was the least effective inhibitor with an estimated IC50 value of 90 µM. We demonstrated the presence of the AKR1C3 protein in most cell lines of hormone-dependent cancer. We have shown that selected nonsteroidal anti-inflamatory drugs and hormonal drugs reduce the activity of the AKR1C3 enzyme and act as inhibitors.

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Study of Estranes as Potential Inhibitors of Enzymes AKR1C1 and AKR1C2

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Chemoresistance poses an important challenge and represents the main cause of unsuccessful cancer treatment. It is a complex process involving various mechanisms that enable cancer cells to resist the cytotoxic effects of chemotherapeutic drugs. Among these, aldo-keto reductase (AKR) enzymes have emerged as important contributors, as they may be directly reducing cellular stress resulting from the action of these agents. Enzymes AKR1C1 and AKR1C2 catalyze the reduction of various endogenous and exogenous compounds, including toxic products resulting from the action of chemotherapeutic agents, and are thus associated with chemoresistance in various cancers. AKR1C1 and AKR1C2 enzymes also catalyze reductions of steroid hormones. Based on this, we hypothesized that newly synthesized steroid compounds from the estrane group inhibit the activity of AKR1C1 and AKR1C2 enzymes. This study aimed to evaluate the potential inhibitory action of a series of 15 estrane compounds and two structural analogs against recombinant AKR1C1 and AKR1C2 enzymes. The activity of recombinant enzymes was determined in the presence of a substrate 2-acenaphthenol and coenzyme NADP+ using a spectrophotometric method by measuring absorbance at 340 nm in the absence and presence of the synthesized estrane compounds. Three compounds achieved inhibition over 40% of the enzyme AKR1C2 at a concentration of 10 μM. We determined the IC-50 value of 7,60 μM for TZ-57, the most potent inhibitor in our study. To further support experimental observations and explore potential binding interactions, molecular docking was performed for the same compound. This study contributes to a better understanding of how newly synthesized compounds affect the enzymatic activity of AKR1C1 and AKR1C2. The best inhibitor represents a lead compound for further structural optimizations, supporting the development of new therapeutic agents that could help overcome chemotherapy resistance.

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AKR1C1-3 Proteins Are Expressed in Model Platinum-Resistant Cell Lines of High-Grade Serous Ovarian Cancer

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Epithelial ovarian cancer is the most common ovarian cancer, with high-grade serous ovarian carcinoma (HGSOC) being the most aggressive subtype. Due to its asymptomatic course and frequent late-stage diagnosis, HGSOC is associated with a poor prognosis and high mortality. Although it initially responds to platinum-based chemotherapy, it often recurs in a chemoresistant form. Chemoresistance is a multifactorial phenomenon associated with altered metabolism of chemotherapeutic agents and cytotoxic products catalysed by the enzymes of aldo-keto reductase (AKR) 1C. Overexpression of AKR1C1–3 is associated with resistance to therapy in various cancers, but its role in HGSOC is still poorly understood. Therefore, this study had two objectives: 1) to evaluate the protein levels of AKR1C1-3 in six HGSOC cell lines (OVSAHO, OVCAR-3, Kuramochi, OVCAR-4, Caov-3 and COV362) that differ in their sensitivity to carboplatin, and 2) to evaluate the effects of prolonged exposure to carboplatin by analysing AKR1C1-3 levels in OVCAR-3 (up to 0.5 µM), OVCAR-4 (up to 0.4 µM and up to 1.2 µM) and Caov-3 (up to 0.8 µM) cells. For Western blot analysis HepG2 was used as positive control, placenta as negative control and HIO-80 as reference for non-cancerous ovarian epithelium. Results showed that AKR1C1/2 was expressed in all HGSOC cell lines, with highest levels in the most carboplatin-resistant lines (Caov-3 and COV362), intermediate levels in OVSAHO, OVCAR-3 and Kuramochi, and low levels in OVCAR-4 and HIO-80. Expression of AKR1C3 followed a similar pattern and increased with carboplatin resistance: lowest in OVSAHO, intermediate in OVCAR-3, Kuramochi and OVCAR-4 and highest in Caov-3 and COV362, while HIO-80 had intermediate levels. With prolonged carboplatin treatment, AKR1C1–3 protein levels generally decreased compared to untreated controls, except for OVCAR-4 at 0.4 µM. These results suggest that AKR1C1–3 are associated with carboplatin resistance and exhibit dynamic, context-dependent regulation in response to chemotherapy.

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Local Androgen and 11-oxyandrogen Signaling as a Prognostic and Therapeutic Axis in High-Grade Serous Ovarian Cancer

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High-grade serous ovarian cancer (HGSOC) is the most lethal gynecological malignancy, exhibiting marked heterogeneity that complicates treatment. The implications of intratumoral androgen metabolism and signaling, particularly involving 11-oxygenated androgens, in HGSOC remains poorly understood. In our study, we analyzed expression of key androgen-metabolizing enzymes and androgen receptor (AR) in relation to tumor site, chemotherapy response, and survival using two public HGSOC cohorts. Quantitative gene expression and steroid metabolism assays upon incubation with classic and 11-oxyandrogen precursors were performed in six HGSOC cell lines and one normal ovarian epithelial cell line. Untargeted transcriptomic and metabolomic profiling were performed to assess cellular responses to potent classic and 11-oxygenated androgens in the AR-positive OVSAHO cell line. We observed differential expression of steroid-metabolizing enzymes and AR between primary and metastatic tumors and chemo-sensitive and chemo-resistant tumors. Furthermore, higher intra-tumoral expression of *HSD11B2*, *HSD17B2*, and AR correlated with improved survival, whereas elevated *PAPSS1/2* and *HSD17B4* predicted poorer outcomes. *In vitro*, classic androgen precursors showed limited conversion to bioactive androgens and did not generate 11-oxyandrogens. In contrast, 11-oxyandrogen precursors were efficiently converted to the potent AR agonist 11KT in chemo-sensitive HGSOC cell lines, but not in chemo-refractory or control lines. Potent classic and 11-oxyandrogens induced stress-adaptive and proliferative transcriptional responses and reduced intracellular amino acid content. These changes were associated with a trend toward reduced cell proliferation. Our findings offer mechanistic insight into local androgen and 11-oxyandrogen metabolism and signalling in HGSOC and reveal steroid-induced cellular vulnerabilities that may synergize with chemotherapy or molecularly targeted therapies. These results further support the therapeutic potential of modulating steroid pathways in HGSOC.

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Molecular Dissection of Advanced-Stage Primary Endometrial Tumors by Mismatch Repair Status Reveals Distinct Transcriptomic Signatures and Actionable Targets

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Endometrial cancer (EC) is one of the most common gynecological malignancies in high-income countries and carries poor outcomes in advanced-stage disease. While the addition of immunotherapy to first-line chemotherapy has significantly improved survival in mismatch repair-deficient (MMRd) advanced EC, over two-thirds of cases are mismatch repair-proficient (MMRp) and respond poorly to this approach. This highlights a major unmet clinical need. Here we compared advanced ECs by mismatch repair status. Clinical and transcriptomic data from the TCGA-UCEC cohort were downloaded using the TCGABiolinks package in RStudio. Patients with primary, advanced-stage EC and available clinical, molecular, and transcriptomic data were included. Differential gene expression between MMRd and MMRp tumors was performed using DESeq2. Cox proportional hazards models were fitted to evaluate associations between levels of differentially expressed genes and disease-specific survival (DSS) in the MMRp subgroup. Of 545 patients with EC, 139 had advanced-stage disease (27 MMRd, 112 MMRp). There was no significant difference in survival between MMRd and MMRp populations. Differential expression analysis ($|\text{fold change}| > 2$, adjusted $p < 0.01$) identified 974 genes. Of these, 268 genes were significantly associated with DSS in the MMRp group. Specifically, genes linked to enhanced immune activation, differentiation-associated signaling, and suppression of proliferative/stemness pathways were associated with better survival. In contrast, higher expression of genes related to neuroendocrine features, stemness, invasion, WNT pathway activation, immune evasion and cellular plasticity correlated with worse outcomes. Advanced MMRp ECs are transcriptionally distinct from advanced MMRd tumors. Within MMRp tumors, we identified gene expression programs associated with prognosis. These findings highlight potential therapeutic targets for this high-risk subgroup and support the development of molecular subtype-adjusted treatment strategies.

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Diagnosis and Prognosis of Hormone Dependent Diseases

Natural Biopsies of the Endometrium: Insights from Menstrual Effluent into Iron-Regulated Migration Pathways in Endometriosis

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A non-invasive diagnostic for endometriosis is a critical unmet need. Menstrual effluent (ME) provides repeatedly accessible cells. We sought to understand the differences in biology of cells in ME of cases versus controls for an ME-based assays.
Primary ME outgrowths from cases and controls were profiled with ferric ammonium citrate (FAC) ± L-glutamine (GLN) and ferroptosis modulators (RSL3/Erastin). Wound migration was tracked longitudinally, proliferation with CyQuant. Live imaging quantified lipid peroxidation (BODIPY-C11) and mitochondrial potential/ROS (MitoTracker/MitoSOX). Secreted/cellular proteins were measured by label-free LC-MS/MS. Single-cell RNA-seq and DNA methylation assessed cellular states and epigenetic features. A confirmatory qPCR/protein panel was summarized as $2^{-}\Delta\Delta Ct$ (cases–controls).
FAC accelerated migration and proliferation preferentially in cases, with early lipid-ROS spikes (<6 h) and recovery of mitochondrial potential/mtROS - evidence of stress tolerance. Cases proliferated more confirmed also by qPCR: MKI67 strongly upregulated with iron (with sustained elevation after prolonged exposure), alongside EMT activation. Chronic FAC induced durable DMRs enriched at WNT/ECM/angiogenesis loci accompanied by persistent transcriptional shifts after washout and partial maintenance of proliferation and pro-migratory state, evidence of iron-encoded "epigenetic hysteresis. Proteomics after FAC in cases revealed increases in ferroptosis, mitochondrial and motility programs, trafficking/stress factors and immune-matrix proteins. GLN sensitized FAC+RSL3/erastin responses in cases, whereas exogenous GSH buffered them. By ~99 h, cases retained enhanced closure across conditions, while CT remained quiescent, consistent with cystine-independent antioxidant compensation in cases and tighter system-Xc^{-} dependence in controls.
ME-based functional/molecular assay distinguishes cases from controls by uncovering iron-driven hyper-motility/proliferation, ferroptosis defenses, and EMT. This non-invasive platform supports diagnostic development and patient stratification for redox/iron-targeted interventions.

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Can ATP7A Transporter Serve as a Biomarker for Predicting Chemoresistance in High-Grade Serous Ovarian Cancer?

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Ovarian cancer (OC), particularly high-grade serous carcinoma (HGSC), is a major cause of gynecological cancer deaths due to late diagnosis and chemoresistance. Up to one-third of patients show primary resistance to platinum-based chemotherapy (Pt-BM), and about 80% develop resistance during treatment, leading to incurable recurrence. Despite extensive research, no validated molecular biomarkers predict platinum resistance. Laboratory studies suggest that ATP7A, a copper (Cu) transporter, is associated with Pt-BM resistance, but clinical validation is lacking. Ceruloplasmin (CP), the main Cu-binding protein in blood, is functionally linked to ATP7A activity.
This study was conducted at the Department of Gynecology, Ljubljana University Medical Center and at Institute for Pharmacology and Experimental Toxicology, Faculty of Medicine. It included in vitro experiments using OAW28 and PEO1 cell lines and a clinical analysis of ascites, blood, and tissue samples from 28 HGSC patients treated with neoadjuvant chemotherapy (NACT). Flow cytometry measured ATP7A expression levels in cell lines. Clinical samples were analyzed for ATP7A gene expression using qPCR and CP concentrations in plasma and ascites using ELISA.
In vitro results showed significantly higher ATP7A expression in the chemoresistant OAW28 cell line. Clinically, 54% of patients had ATP7A expression in tissues before NACT, and 43% after NACT. Higher ATP7A expression in the peritoneum before NACT correlated with a poor CA-125 KELIM score. Patients with omental ATP7A expression had elevated plasma CP levels before treatment. CP levels dropped after NACT, and higher post-treatment CP was linked to shorter platinum-free intervals.
Coclusion: Together, these findings suggest that ATP7A and CP could serve as potential biomarkers for platinum resistance, pending further validation.

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Multi-steroid Profiling and Machine Learning Reveal Androgens as Promising Biomarker Candidates for Endometrial Cancer Diagnosis

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Endometrial cancer (EC) is the most frequently diagnosed gynecologic malignancy in developed countries. A histologically confirmed diagnosis, ideally obtained from a surgical specimen or image-guided biopsy of treatment-naïve tumor tissue, is essential for appropriate management. However, because such procedures are invasive, there is a pressing need for accurate and less-invasive diagnostic approaches. In our study, we aimed to evaluate the diagnostic potential of preoperative serum steroid levels in EC alone and in combination with clinical parameters and biomarkers CA-125 and HE4. We included 62 patients with EC and 70 controls with benign uterine conditions who underwent surgery between June 2012 and February 2020. Preoperative serum levels of classic androgens, 11-oxyandrogens, glucocorticoids, and mineralocorticoids were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Machine learning was used to assess their diagnostic and prognostic value alone and combined with clinical parameters and biomarkers. Patients with EC had significantly higher serum levels of classic androgens (androstenedione, testosterone), 11-oxyandrogens (11β-hydroxy-androstenedione, 11β-hydroxy-testosterone), and glucocorticoids (17α-hydroxy-progesterone, 11-deoxycortisol) compared to controls. While individual steroids had limited diagnostic value, a multivariate model including classic androgens, CA-125, HE4, BMI, and parity achieved an AUC 0.87, 79.1% sensitivity and 74.7% specificity in distinguishing EC from benign uterine condition. This model outperformed our previously published model based on CA-125, HE4, and BMI (AUC: 0.81, $p < 0.0001$). In conclusion, patients with EC exhibit distinct steroid hormone profiles compared to controls. While steroids alone offer modest diagnostic and prognostic value, integrating them into multivariate models improves diagnostic accuracy.

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Proteomic Profile of Uterine Cavity Lavage in Endometriosis Patients - Promising Approach on Biomarker Discovery and Pathophysiology Explanation

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Endometriosis is a prevalent benign gynecological condition, impacting up to 10% of all women. While endometriosis cause, pathophysiology and its impact on infertility remains unclear, our aim was to evaluate the protemic changes in uterine cavity of patients with endometriosis using uterine cavity lavage sample analysis and to find proteins associated with pathophysiolgical pathways of endometriosis and impaired fertility.

The study involved the protein microarray method, determining levels of proteins in uterine cavity flushing from 13 patients with endometriosis and the control group. Samples were collected according to SOP. Samples were analyzed in dual-color antibody microarrays targeting 1,438 different proteins with 1,920 antibodies. Between case samples and control samples, three antibodies recorded differential protein abundance. Proteins with differential abundance are B- and T-lymphocyte attenuator (BTLA), Keratin type II cytoskeletal 8 (K2C8) and Cathelicidin antimicrobial peptide (CAMP).

BTLA functions as inhibitory receptor on lymphocytes that negatively regulates antigen receptor signaling via PTPN6/SHP-1 and PTPN11/SHP-2. This upregulation leads to the inflammatory activation of these cells and promotes macrophage migration and the release of inflammatory cytokine.

Cytoskeletal proteins like K2C8, play role in regulating and organizing cell division, which holds immense significance in the context of endometriosis, condition characterized by the growth of endometrium-like tissue outside the uterus.

CAMP acts via neutrophil N-formyl peptide receptors to enhance the release of Chemokine (C-X-C motif) ligand 2 (CXCL2). CXCL2 influences the proliferation, migration, angiogenesis, invasion and fibrosis of endometriotic cells.

Uterine cavity lavage proteomic profiling is a viable, less invasive diagnostic method for identifying endometriosis. Development of proteomic identification techniques, gives us the possibility of identifying a wider specter of proteins in endometrial fluid in patients with endometriosis. The results described provide new endometriosis-related proteins, to be validated in larger cohort as diagnostic markers, also to help understand endometriosis pathophysiology and its impact on infertility.

Funding: This study was funded by ARIS grant P3-0449 to T.L.R.

AKR1C3 as a Target for New Treatment Options in Endometriosis

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Endometriosis is a chronic disease characterized by the presence of endometrial-like tissue outside the uterus. Due to the complexity of the disease, there is currently no cure, and the diagnosis is often delayed. In addition to surgical treatment, current medical therapies include hormonal drugs: progesterone receptor agonists, oral contraceptives (off-label) and GnRH agonists/antagonists, all of which impair fertility, as well as non-steroidal anti-inflammatory drugs, which also have serious side effects with prolonged use. New medical therapies should focus on the treatment of endometriosis without affecting the fertility of these patients. The enzyme AKR1C3 plays a central role in intra-tissue estrogen, androgen and prostaglandin biosynthesis and could represent a target for new treatment options. The aim of this study was to investigate the levels of the enzyme AKR1C3 in endometriotic tissue from 84 patients with different types of endometriosis as an extension of a previous pilot study. Samples from patients with ovarian, peritoneal, deep endometriosis and deep endometriosis combined with ovarian or peritoneal endometriosis were used for immunohistochemical (IHC) staining with validated antibodies. Several categorical variables were considered, such as age, BMI, menstrual phase, rAFS stage (revised American Fertility Society classification system), menstrual pain score, peroral contraception and hormonal therapy. The intensity and perimeter of AKR1C3 IHC staining in the tissue samples were measured, and a normalized value was determined for each sample by calculating the ratio of IHC intensity to IHC perimeter. Based on IHC data, Spearman's correlations were calculated between numerical and categorical numerical features. For comparison of the distributions of IHC intensity, perimeter, and their ratio across different categorical variables, Wilcoxon-Mann-Whitney test was used. A pilot study of 37 samples showed that AKR1C3 IHC intensity was influenced by the variables examined, and we found a correlation with rAFS stage, use of peroral contraceptives in the last three months, use of hormone therapy in the past, infertility reported as the reason for surgery, and menstrual pain score. However, the analysis of a further 47 samples is currently underway.

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Potential of Soluble Immune Checkpoints as Prognostic and Predictive Biomarkers in Endometrial Cancer

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Soluble immune checkpoints (sICs) have recently been studied as diagnostic, prognostic and predictive biomarkers in a range of cancers. Aim of presented study was to discover potential diagnostic, prognostic and predictive biomarkers among a set of sICs in endometrial cancer (EC). We included 50 patients diagnosed with EC prior to surgery and 26 women undergoing surgery for benign gynaecologic conditions as controls. We measured plasma concentrations of 16 sICs using Luminex XMAP Multiplex immunosorbent assay, 8 inhibitory (sPD-1, sPD-L1, sPD-L2, sCTLA-4, sLAG3, sTIM3, sBTLA, sHVEM), 6 stimulatory (sICOS, sGITR, sGITR-L, sCD40, sCD27, sCD2) and 2 with mixed role (sCD80, sCD86). Study cohort included 66% of EC patients who had non-aggressive histological type, while 33% of tumors exhibited MMR deficiency and 14% had aberrant p53 expression. Majority of tumors were confined to the uterus, with 16% of patients having stage cancer of stage IIIA or higher. sICs levels did not significantly differ between control patients and EC patients. Also, sICs levels did not significantly differ by aggressiveness of histological type. Levels of sTIM3 tended to be higher in more advanced stage cancers, similarly, its levels were higher in higher risk group patients. Levels of sPD-1, sPD-L1, sLAG-3, sICOS, sGITR, sGITRL, sCD86 were higher in MMR deficient tumors. Contrary to published findings in a small cohort of EC patients and in other malignancies, we did not detect differing sICs levels in EC patients against patients without cancer. This may be due to majority of the patients in presented study having localised disease. Further studies are needed before ruling out sICs as diagnostic biomarkers in endometrial cancer. Biological foundation of higher levels of some sICs in MMR deficient tumors may be their immunoreactivity, stimulating immune response. This finding may be clinically significant, as blood-based prognostic and predictive biomarkers could preclude the need for repeat biopsies and overcome tissue biomarker limitations.

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Biological Variation and Intercorrelation of Soluble Immune Checkpoints in Healthy Individuals

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Soluble immune checkpoints (sICs), related to their transmembrane counterparts targeted in cancer immunotherapy, are measurable in peripheral blood and were previously studied as potential biomarkers. However, data on sICs' levels biological variation in healthy individuals is scarce. Limited research suggests correlations among different sICs levels, likely due to their shared roles in immune regulation. In this study, we included 26 women undergoing surgery for benign gynaecologic conditions, obtained their peripheral blood plasma samples by standardised protocol and measured levels of 16 sICs using the Luminex xMap assay. sICs levels were within assay's detectable range except for one measurement for PD-1, PD-L1, BTLA, CD28, CD86. Inter-individual coefficient of variation was below 30% for PD-L2, TIM3, CD40, varying between 30% and 66% for others. None of the sICs levels differed by menopausal status and only PD-L2 levels moderately positively correlated with age. Levels of PD-1, PD-L1, LAG-3, BTLA, GITR, GITLR and CD28 were moderately positively correlated with body mass index (BMI). We identified a group of sICs whose levels were strongly positively linearly correlated, namely PD-1, PD-L1, CTLA-4, LAG-3, BTLA, ICOS, GITR, GITR-L, CD28, CD80, CD86. Other two identified groups of intercorrelated sICs were TIM-3, CD40 and HVEM, CD27, respectively. Plasma levels of sICs in control group of patients are within detectable ranges of modern analytical methods enabling their study in health and disease. Association of elevated levels of some iICs with high BMI may be due to systemic inflammation present in obese individuals. Nevertheless, one needs to consider accounting for this association in further studies of sICs as biomarkers in patients. Information on sICs intercorrelation can be used to economise further studies by limiting the number of measured and studied sICs. Presented observations need to be confirmed in a larger study of healthy individuals before considering specific sICs in further biomarker studies on patients.

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Whole-Blood RNA-seq and Machine Learning Identify Candidate Biomarkers for Peritoneal Endometriosis

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Endometriosis is a chronic gynecological condition affecting up to 11% of women of reproductive age. Diagnosing peritoneal endometriosis (PE) remains challenging, as it cannot be detected by standard imaging. Although numerous biomarkers of endometriosis have been proposed, none were clinically validated. However, recent advances in transcriptomics offer new opportunities for biomarker discovery. This study aimed to identify novel blood biomarkers for PE using whole blood RNA sequencing with machine learning approaches. Women undergoing laparoscopic surgery for suspected endometriosis were enrolled and categorized with the presence of PE (n=20), PE with ovarian endometriosis (PE+OE, n=8), or absence of endometriosis (controls, n=20). Whole-genome RNA sequencing was performed on patients whole blood samples. Differentially expressed genes (DGEs) and transcripts (DTEs) were identified (FDR<0.05). RNA sequencing data underwent standard bioinformatics processing, including read trimming, quality control, alignment, quantification, differential expression, and functional enrichment analysis. The processed data were then integrated into a machine learning pipeline: feature selection reduced the dataset to a minimal subset of genes or transcripts, which was used to train an SVM classifier to distinguish patients with and without endometriosis. In the proliferative phase, no DGEs and only two DTEs differentiated PE from controls, whereas in the secretory phase, 1,035 DGEs and 922 DTEs were identified. No overlapping DGEs/DTEs were found between phases. PCA separated samples by menstrual phase only. Functional enrichment analysis performed on secretory phase samples showed that upregulated DGEs between control and endometriosis patients were enriched in angiogenesis, lipopolysaccharide binding and immune receptor activity, reflecting roles in vascular development and innate immune responses. A set of six transcripts identified via feature selection yielded the best SVM performance (ROC AUC=0.92, sensitivity=75%, specificity=100%). This is the first study to use whole genome RNA sequencing to discover blood biomarkers for PE. Validation of the selected DTEs in a larger cohort is currently underway.

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Combined Models with Plasma Phosphatidylcholines, Amino Acids, TGFBI, and Clinical Data Distinguish Patients with Peritoneal Endometriosis from Control Patients

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Endometriosis is a complex, benign gynaecological disease that affects up to 10% of women of reproductive age and is associated with severe pain and infertility. Although ovarian and deep endometriosis can be diagnosed by imaging techniques, peritoneal endometriosis is still dependent on surgical diagnosis. The aim of this study was to construct combined diagnostic models for endometriosis, with a focus on peritoneal endometriosis, based on targeted metabolomics, single protein data and clinical data. A prospective case-control study included a total of 513 patients, 316 patients with different types of endometriosis and 197 control patients from the University Medical Centre Ljubljana, Slovenia, and the University Hospital Vienna, Austria. The discovery phase comprised a total of 278 patients and the validation phase a total of 235 patients. Plasma samples were collected according to a strict standard operating procedure and 163 metabolites were measured by LC-MS/MS. Protein TGFBI was measured by ELISA. The classification models were created using logistic regression. Five models including two metabolite ratios, presence of pain and/or infertility and levels of protein TGFBI distinguished patients with peritoneal endometriosis from control patients with an AUC of 0.925 - 0.951 in discovery phase and an AUC of 0.878 - 0.895 in the validation phase. The best performing model based on combined metabolomics data and TGFBI levels showed an AUC of 0.925 and 0.895 in discovery and validation phases, respeectively. The best validated model shows a sensitivity of 84.6 % and a specificity of 79.8 %. These models have been validated in an independent cohort and two of these models meet the criteria for a triage. However, further multicenter validation in a larger number of patients with peritoneal endometriosis is still needed. This study shows that models containing a combination of different molecules, metabolites and proteins with clinical data have better properties than metabolites alone.

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Serum Steroid-Protein Panels for Differentiating Ovarian Cancer from Non-Malignant Adnexal Masses

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Ovarian cancer is the deadliest gynecological malignancy, largely due to the advanced stage at diagnosis in most patients. This study investigates whether systemic steroids can serve as biomarkers to distinguish malignant ovarian tumors from non-malignant adnexal masses. We preformed prospective, single-center observational study including 99 women with adnexal masses who underwent surgery between December 2021 and February 2025. Preoperative serum levels of 17 steroid hormones were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Machine learning was employed to assess the diagnostic potential of these steroids in distinguishing ovarian cancer (n=43) from non-malignant adnexal masses (n=56). Patients with ovarian cancer had lower levels of 11β-hydroxy-testosterone (11OHT), 11-keto-testosterone (11KT), and testosterone compared to those with non-malignant adnexal masses. Using stepwise feature selection, we developed two diagnostic models incorporating three 11-oxyandrogens (11KT, 11OHT, and 11β-hydroxy-androstenedione), patient age, and either cancer antigen 125 (CA-125) or human epididymis protein 4 (HE4) for distinguishing malignant from non-malignant adnexal masses. The model including CA-125 achieved AUC of 0.907, 88.9% sensitivity and 82.0% specificity, while the model including HE4 achieved AUC of 0.911, 94.4% sensitivity and 77.3% specificity as evaluated by cross-validation. Both models significantly outperformed CA-125, HE4, and the Risk of Ovarian Malignancy Algorithm (ROMA) index alone. In conclusion, patients with ovarian cancer exhibit distinct steroid profiles compared to those with non-malignant adnexal masses. If validated, the models could enhance diagnosis, reducing unnecessary surgeries for benign conditions while ensuring timely treatment for ovarian cancer, particularly when conventional biomarkers are inconclusive.

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Endometrial Cancer Diagnostic Models Using Clinical and Lifestyle Data

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Endometrial cancer (EC) is the most common gynaecological malignancy in industrialized countries. Currently, diagnosis and treatment rely on histopathological and surgical findings. The ERA-NET Transcan2 project "Biomarkers for Diagnosis and Prognosis of Endometrial Carcinoma" (BioEndoCar; NCT03553589), coordinated by the University of Ljubljana, addressed the need to find a valid, non-invasive diagnostic or prognostic method. A prospective observational case-control study was conducted at six medical centers across Europe. Plasma samples from women diagnosed with EC and control subjects were analysed using non-targeted/targeted metabolomic and semi-quantitative immune-based proteomic approaches. In addition, we collected a wealth of data ranging from clinical data to detailed lifestyle data. The aim of this study was to create diagnostic/prognostic models based on preoperatively collected clinical data and epidemiological data.
The BioEndoCar cohort comprised over 440 patients for whom more than 300 variables were collected using electronic case report forms. Using the expert opinion, we focused on 56 clinical and lifestyle data from demographics, medical history, current and past medications, food supplements, physical examination and physical activity segments of the BioEndoCar dataset, excluding blood sampling, surgical procedures and pathology groups and constructed diagnostic models using logistic regression. As expected, the computed models showed good discriminatory abilities. The endometrial thickness single-variable model had an average AUC value of over 0.86, with an average sensitivity and average specificity of nearly 80%. The average AUC values increased with the number of included variables. The eight-variable model comprising of endometrial thickness, postmenopausal status, infertility, bleeding symptoms, BMI, parity, pelvic abnormality and menarche showed an average AUC of 0.91 with an average sensitivity of 88% and average specificity of 81% We stabilized the computed metrics using multi-iterated bootstrap resampling with replacement methods. The results of this multicenter study show that models based on preoperatively collected clinical data and epidemiological data can contribute to the diagnosis of EC.

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Research on Hormone Dependent Diseases – Patients' Perspectives

Living with Endometriosis: Perspectives of Those with Lived Experience

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Endometriosis represents a long-term and complex challenge that goes beyond a medical diagnosis. Chronic pain, fertility issues, prolonged diagnostic delays, and uncertainties related to treatment effectiveness significantly affect not only the patients' physical but also emotional and social lives. The condition impacts educational and career opportunities, as well as personal relationships and self-image, with patients often facing the invisibility of their experiences and a lack of understanding from both the professional and wider society, as well as loved ones. Despite this, they develop coping strategies and resilience, while seeking support that enables them to maintain quality of life. Thus, endometriosis is not merely a health issue but a life-long challenge that requires a holistic approach, empathy, and support within both the healthcare system and the broader social context. Research on conditions like endometriosis offers hope for a better quality of life in the future – whether through new diagnostic procedures that allow for faster and less invasive detection, or through novel treatment methods. Above all, those affected emphasize the importance of including their everyday experiences in research, calling for interdisciplinary collaboration in the study of the condition.

Endozavest – Endometriosis Society of Slovenia is a non-governmental organization that brings together women with lived experience of endometriosis as well as experts from various fields. Its mission is to raise public awareness about endometriosis, provide support to those affected and their families, and advocate for a comprehensive approach to the disease. Through lectures, workshops, and awareness campaigns, Endozavest contributes to greater recognition of endometriosis and to reducing the stigma surrounding it. Endozavest's vision is to create a social environment in which women with endometriosis have access to a timely diagnosis, appropriate treatment, understanding, and equal opportunities for full participation in society.

Funding: /

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