

**Therapeutic Validation of Dopamine Receptor 2 (DRD2)
Inhibition in Gynaecological Carcinomas Using Advanced 3D
Organotypic Tumour Slice Models**

Maria Charlotte Edwards

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DOCTORAL THESIS

Therapeutic Validation of Dopamine Receptor 2 (DRD2)
Inhibition in Gynaecological Carcinomas Using
Advanced 3D Organotypic Tumour Slice Models

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“At the heart of science is an essential balance between two seemingly contradictory attitudes—an openness to new ideas...and the most ruthless sceptical scrutiny of all ideas, old and new.”

—Carl Sagan

Table of Contents

Summary	i
Resum	iii
List of Abbreviations.....	v
Chapter 1 Introduction.....	1
Section 1.1 Overview of Gynaecological Cancers and the Current Therapeutic Landscape	3
1.1.1 Gynaecological Cancers: An Overview	4
1.1.2 Ovarian Cancer	5
1.1.3 Further Classification of HGSOE	7
1.1.4 Endometrial Cancer.....	9
1.1.5 Further Classification of Endometrial Cancer	11
1.1.6 Standard-of-Care and Targeted Therapy	12
1.1.7 Drug Discovery and Drug Repurposing.....	13
Section 1.2 The Tumour Microenvironment and Therapeutic Resistance	17
1.2.1 Tumour Vasculature	19
1.2.2 Stroma and Extracellular Matrix	21
1.2.3 Tumour Immunological Landscape	24
1.2.4 Tumour Infiltrating Lymphocytes (TILs)	25
1.2.5 Myeloid Compartment.....	28
1.2.6 Cancer Stem Cells.....	31
1.2.7 Tumour Infiltrating Nerves.....	32
Section 1.3 Preclinical Models for Drug Discovery in Gynaecological Cancer and Their Limitations	33
1.3.1 <i>In vitro</i> : Cell Culture.....	35
1.3.2 3D models: Spheroids, Organoids, Tumour-on-chip, and Bioprinted Models.....	36
1.3.3 <i>In vivo</i> Animal Models	38
Section 1.4 3D <i>Ex vivo</i> Organotypic Tumour Slice Culture (OTSC) and Their Application in Gynaecological Cancer	41
1.4.1 OTSC and the TME	43
1.4.2 OTSC Technical Considerations.....	44
1.4.3 OTSC in Gynaecological Cancer Drug Evaluations	46
Section 1.5 Dopaminergic Signalling Pathways: Cancer Progression and Immunomodulation	49
1.5.1 Dopamine Receptor Signalling	50
1.5.2 D1-like Signalling.....	51

1.5.3 D2-Like signalling:	52
1.5.4 β -arrestin-dependent signalling (alternative pathway).....	52
1.5.5 Dopaminergic Dysregulation in Gynaecological Cancer Progression	53
1.5.6 DRD1 Agonism and DRD2 Antagonism in Gynaecological Cancer and the TME	55
1.5.7 Dopaminergic Immunomodulation.....	56
Section 1.6 Nanotherapeutic Strategies in Gynaecological Cancer	59
1.6.1 Nanoparticles	60
1.6.2 Nanomedicines for Gynaecological Cancers	61
1.6.3 Polymeric Nanoparticles	63
Section 1.7 Aims and Hypotheses.....	65
1.7.1 Aims	66
1.7.2 Hypotheses	66
Chapter 2 Development of OTSC Models of Ovarian and Endometrial Cancer and their Validation with Chemotherapy	67
2.1 Methods.....	69
2.1.1 Cell Culture.....	69
2.1.2 Xenograft Implantation	69
2.1.3 Optimisation of Organotypic Tumour Slice Culture	70
2.1.4 OTSC Cell Culture	72
2.1.5 Haematoxylin & Eosin (H&E)/ Immunohistochemistry	73
2.1.6 Digital Quantification and Macro Scripting.....	74
2.1.7 IHC of Slices Treated with Chemotherapy.....	76
2.1.8 Viability Assessment (AlamarBlue)	76
2.1.9 Cytotoxicity Monitoring (LDH Cytotoxicity Assay)	77
2.1.10 Cell Population Composition Analysis	78
2.1.11 Tumour Dissociation.....	78
2.1.12 Flow Cytometry (Tumour).....	79
2.1.13 Tumour Dissociation of OTSC Slices.....	79
2.1.14 Flow Cytometry (OTSC).....	79
2.1.15 Validation with Chemotherapy	80
2.1.16 AlamarBlue and LDH Assays in Cell Lines with Chemotherapy	80
2.1.17 AlamarBlue and LDH Assays in Slices with Chemotherapy	81
2.1.18 Statistical Analysis	81
2.2 Results	83
2.2.1 OVCA8 and ISHIKAWA Slices Retain Metabolic Viability up to Day 4 in Culture.....	83
2.2.2 OTSC Platform Successfully Recapitulates Native TME Heterogeneity.....	87

2.2.3 OVCAR8 Slices Exhibit Cytotoxicity to Chemotherapy	88
2.2.4 ISHIKAWA Slices Exhibit Resistance to Standard Chemotherapy	91
Chapter 3 Characterising the Dopaminergic Interactome in Ovarian and Endometrial Cancer Cell Lines and the Screening of Dopaminergic Modulators ...	95
3.1 Methods.....	97
3.1.1 Cell Culture.....	97
3.1.2 Reverse Transcription qPCR.....	97
3.1.3 ELISA	98
3.1.4 Bradford Assay	99
3.1.5 Protein Extraction for Western Blot.....	99
3.1.6 SDS-PAGE and Western blotting	100
3.1.7 Flow Cytometry for DRD1 and DRD2 Protein Expression.....	101
3.1.8 High-Throughput Screening of Dopaminergic Receptor Modulators	103
3.1.9 Mechanism of Domperidone: Endocytic Inhibitors	104
3.1.10 Wound-Healing Migration Assay.....	105
3.1.11 Statistical Analysis	106
3.2 Results	107
3.2.1 DRD2 is the Predominant Dopamine Receptor Across HGSOC and EC Models	107
3.2.2 DRD1 Expression Prevalence Increases in the OVCAR8 OTSC Model.....	108
3.2.3 DRD2 Blockades Identified as a Promising Anti-Tumour Strategy.....	110
3.2.4 Domperidone Efficacy not Affected by Endocytosis Blockades.....	113
3.2.5 Targeted DRD2 Inhibition Significantly Impairs Endometrial Cancer Motility	116
3.2.6 OVCAR8 and ISHIKAWA Cells Exhibit Opposing Signalling Responses to DRD2 Antagonism.	120
Chapter 4 The Efficacy of DRD2 Blocker Domperidone in ex vivo Ovarian and Endometrial OTSC Models and Patient-Derived Biopsy Slices.....	123
4.1 Methods.....	125
4.1.1 2D Monoculture Viability and Cytotoxicity Assays	125
4.1.2 OTSC and Real-Time and Fixed Time-Point Viability Assessments	125
4.1.3 Quantification of OTSC Slice Gene Expression using TLDA	125
4.1.4 LIVE/DEAD Kit Staining and Confocal Microscopy	126
4.1.5 Flow Cytometry (Population Composition and Immune Subsets)	127
4.1.6 Patient-Derived OTSC of Ovarian Cancer Biopsy.....	128
4.1.7 Survival Analysis of DRD2 Expression	129
4.1.8 Statistical Analysis	129
4.2 Results	131

4.2.1 DRD2 Antagonism Significantly Reduces Viability in OVCAR8 OTSC Slices	131
4.2.2 DRD2 Antagonism Increases Apoptosis in OVCAR8 OTSC Slices	133
4.2.3 Endometrial OTSC Model Exhibits Potent Resistance to Domperidone	135
4.2.4 Confocal Assessment Confirms Selective Cell Death Following DRD2 Blockade ..	138
4.2.5 DRD2 Inhibition Differentially Modulates Immune Subsets in OTSC Models.....	142
4.2.6 DRD2 Blockade Upregulates the Transcription of Stemness Genes	147
4.2.7 Proteomic Analysis Reveals Potent MEK inhibition and DRD1 Compensation.....	152
4.2.8 Patient Biopsy Pilot Study Validates Heterogeneous Responses to Domperidone .	154
4.2.9 High DRD2 Predicts Opposing Survival Outcomes in Gynaecological Cancer.....	159
Chapter 5 The Development of Domperidone-Loaded Poly(Lactic-co-Glycolic Acid) Nanoparticles and Their Efficacy in Gynaecological Cell Lines and OTSC	161
5.1 Methods.....	163
5.1.1 Nanoparticle Synthesis.....	163
5.1.2 Nanoparticle Characterisation.....	165
5.1.3 Quantification of Domperidone in PLGA-PEG NPs by LC-MS/MS	166
5.1.4 2D Cell Line Viability Assays with Domp-PLGA-PEG NPs	166
5.1.5 OTSC Viability Assays with Domp-PLGA-PEG NPs.....	167
5.1.6 Study of Domp-PLGA-PEG NPs Internalisation and Biodistribution	167
5.2 Results	169
5.2.1 Variable Size and Concentration of Domp-PLGA-PEG NPs	169
5.2.2 Optimised Filtration Protocols Enhance Nanocarrier Homogeneity	170
5.2.3 Domp-PLGA-PEG NPs Reduce Viability of ISHIKAWA Cell Line	173
5.2.4 Negligible Efficacy of Domp-PLGA-PEG NPs in OVCAR8 OTSC	174
5.2.5 Domp-PLGA-PEG NPs Paradoxically Increase ISHIKAWA Slice Viability	177
5.2.6 Confocal Tracking Confirms Time-Dependent Intracellular NP Accumulation.....	177
5.2.7 Confocal Tracking Confirms Multicellular Uptake of NP in 3D Slice Model	180
Chapter 6 Discussion	183
6.1 Development of the OTSC Model: Viability.....	185
6.2 Development of the OTSC Model: TME Heterogeneity	186
6.3 Efficacy of DRD2 Blocking (Domperidone) in Gynaecological Cancers.....	188
6.4 Signalling Modulation by DRD2 Blocking (Domperidone).....	190
6.5 Compensatory Mechanisms	191
6.6 Domperidone and TME Effects	193
6.7 Domperidone Loaded PLGA-PEG Nanoparticles.....	196
6.8 Limitations and Future Directions	198

Chapter 7 Conclusions	199
References	203
Scientific Contributions	235
Scientific Publications (See Annex)	236
Conference Contributions and Posters	236
Training and Academic Activities	236
Awards and Grants	237
Annexes.....	239

Summary

Treatment options for patients with high grade serous ovarian cancer (HGSOC) and Endometrial cancer (EC), particularly those with treatment-resistance and distal metastases, are extremely limited, resulting in very poor outcomes. Therefore, novel therapeutics are urgently needed for these malignancies. Unfortunately, many promising drug candidates in preclinical trials often fail at later evaluation stages. This is in part due to poor drug screens in models without the native tumour composition and TME crosstalk that greatly influences resistance mechanisms and drug response. It is critical that models which better replicate the native environment are used during drug discovery pipelines. In this thesis, we introduce organotypic tumour slice culture (OTSC) models as an innovative drug screening platform; this model is established from ultra-thinly slicing xenografted animal material or patient biopsy within a few hours of resection. These slices maintain viability up to 7 days in culture as well as the structural complexity and cellular heterogeneity of the native tumour, making it an ideal preclinical drug screening model.

Another objective of this thesis is to explore neuromodulating pathways as an anti-cancer strategy. Recently, there has been an increasing amount of evidence reporting dopamine receptor targeting as a new anti-tumour strategy; studies have repurposed dopamine 1 receptor (DRD1) agonists and DRD2 antagonists and indicated that they have antiproliferative and immune-activating effects in *in vitro* and *in vivo* cancer models. However, further investigations are required to validate the efficacy of this strategy in gynaecological models of HGSOC and low-grade EC. Moreover, there is an absence of studies evaluating neuromodulation in *ex vivo* 3D organotypic gynaecological OTSC models which incorporate the 3D tumour architecture and the tumour microenvironment (TME) with its heterogenic cell populations (epithelial, endothelial and leukocyte cells) – all of which play a vital role in the efficacy of anti-cancer drugs. Thus, we conducted a drug screen of DRD1 agonists and DRD2 antagonists in cell lines of HGSOC and EC, identifying one very promising DRD2 antagonist, Domperidone, with repurposing potential. We then carried out a comprehensive exploration of the anti-cancer and

immunomodulatory effects of Domperidone in 3D OTSC models of HGSOc and EC, as well as patient-derived models of ovarian cancer biopsy.

Lastly, this thesis aimed to explore the incorporation of DRD2 antagonist Domperidone in a nano-formulation. In the last decade, nanomedicines – small molecules (1-300nm) manipulated to have a variety of shapes, sizes, and physicochemical properties – have become increasingly popular in anti-cancer formulations, with many receiving clinical approval for the treatment of cancer. One attractive quality of nanoparticles is that they can coat and protect a drug, altering its pharmacokinetic profile and preventing its early degradation prior to reaching the tumour site. Likewise, nanomaterials can be conjugated to cancer-specific agents to form a tumour targeting strategy, while their small size facilitates their tumour uptake for efficacious targeting. Domperidone is a candidate for a nano-formulation due to its low aqueous solubility, low oral bioavailability and reported off-target accumulation and cardiotoxicity. However, to date, there have been no studies investigating a DRD2 blocking nanotherapeutic approach towards gynaecological tumours and their microenvironment. Thus, the use of DRD2-modulating nanoparticles may provide new avenues for the treatment of most prevalent ovarian and endometrial cancers.

Resum

Les opcions de tractament per a pacients amb càncer d'ovari serós d'alt grau (HGSOC) i càncer d'endometri (EC), especialment aquells amb resistència al tractament i metàstasis distals, són extremadament limitades, fet que comporta uns resultats clínics molt deficients. Per tant, es necessiten urgentment noves teràpies per a aquestes malalties. Malauradament, molts candidats a fàrmacs prometedors en assajos preclítics solen fracassar en etapes posteriors d'avaluació. Això es deu, en part, a cribratges de fàrmacs deficients en models que no tenen la composició tumoral nativa ni la interacció amb el microambient tumoral (TME), factors que influeixen enormement en els mecanismes de resistència i la resposta al fàrmac. És crític que s'utilitzin models que repliquin millor l'entorn natiu durant els processos de descobriment de fàrmacs.

En aquesta tesi, introduïm els models de cultiu de talls tumorals organotípics (OTSC) com una plataforma innovadora de cribratge de fàrmacs; aquest model s'estableix a partir de talls ultrafins de material animal xenoinjertat o biòpsies de pacients a les poques hores de la resecció. Aquests talls mantenen la viabilitat fins a 7 dies en cultiu, així com la complexitat estructural i l'heterogeneïtat cel·lular del tumor natiu, cosa que els converteix en un model ideal de cribratge preclínic.

Un altre objectiu d'aquesta tesi és explorar les vies neuromoduladores com a estratègia anticancerígena. Recentment, ha augmentat l'evidència que assenyala el receptor de dopamina com una nova diana antitumoral; diversos estudis han reposicionat agonistes del receptor de dopamina 1 (DRD1) i antagonistes del DRD2, indicant que tenen efectes antiproliferatius i d'activació immunitària en models de càncer *in vitro* e *in vivo*. Tanmateix, calen més investigacions per validar l'eficàcia d'aquesta estratègia en models ginecològics d'HGSOC i EC de baix grau.

A més, hi ha una absència d'estudis que avaluïn la neuromodulació en models ginecològics *ex vivo* 3D de tipus OTSC, els quals incorporen l'arquitectura tumoral tridimensional i el TME amb les seves poblacions cel·lulars heterogènies (cèl·lules epitelials, endotelials i leucòcits), tots ells amb un paper vital en l'eficàcia dels fàrmacs anticancerígens. Així doncs, vam realitzar un cribratge de fàrmacs d'agonistes de DRD1 i antagonistes de DRD2 en línies cel·lulars d'HGSOC i EC, identificant un antagonista de

DRD2 molt prometedor, la Domperidona, amb potencial de reposicionament. Posteriorment, vam dur a terme una exploració exhaustiva dels efectes anticancerígens i immunomoduladors de la Domperidona en models 3D OTSC de HGSOc i EC, així com en models derivats de biòpsies de pacients amb càncer d'ovari.

Finalment, aquesta tesi pretén explorar la incorporació de l'antagonista de DRD2 Domperidona en una nanoformulació. En l'última dècada, les nanomedicines — molècules petites (1-300 nm) manipulades per tenir una gran varietat de formes, mides i propietats fisicoquímiques— han esdevingut cada cop més populars en formulacions anticancerígenes, i moltes han rebut aprovació clínica per al tractament del càncer. Una qualitat atractiva de les nanopartícules és que poden recobrir i protegir un fàrmac, alterant el seu perfil farmacocinètic i evitant la seva degradació prematura abans d'arribar al lloc del tumor. Així mateix, els nanomaterials es poden conjuguar amb agents específics del càncer per formar una estratègia de focalització tumoral, mentre que la seva petita mida facilita l'absorció tumoral per a una diana eficaç.

La Domperidona és una candidata per a una nanoformulació a causa de la seva baixa solubilitat aquosa, baixa biodisponibilitat oral i l'acumulació no desitjada (*off-target*) i cardiotoxicitat documentades. No obstant això, fins a la data, no hi ha hagut estudis que investiguin un enfocament nanoterapèutic de bloqueig de DRD2 cap als tumors ginecològics i el seu microambient. Per tant, l'ús de nanopartícules moduladores de DRD2 pot oferir noves vies per al tractament dels càncers d'ovari i d'endometri més prevalents.

List of Abbreviations

AB: AlamarBlue

ADC: Antibody Drug Conjugate

ANS: Autonomic nervous system

APC: Antigen presenting cell

ATCC: American Type Culture Collection

ATP: Adenosine triphosphate

AUC: Area under the curve

BSA: Bovine serum albumin

CAF: Cancer associated fibroblast

cAMP: Cyclic adenosine monophosphate

cCasp3: Cleaved-caspase-3

CDX: Cell-line derived xenograft

CK-1: Cytokeratin-1

COMT: Catechol-O-methyltransferase

CRAd: Conditionally replicative adenovirus

CSC: Cancer stem cell

Ct: Cycle threshold

DA: Dopamine

DLS: Dynamic light scattering

DR: Dopamine receptor

DRD1–5: Dopamine receptor D1 through D5

EC: Endometrial cancer

ECM: Extracellular matrix

EGF: Epidermal growth factor

ELISA: Enzyme-linked immunosorbent assay

EMT: Epithelial-mesenchymal transition

EOC: Epithelial ovarian cancer

EPCAM: Epithelial cell adhesion molecule

EPR: Enhanced permeability and retention effect

ER: Oestrogen receptor

FAP: Fibroblast activation protein

FBS: Foetal bovine serum

FDA: Food and Drug Administration

FGF: Fibroblast growth factor

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

GEMM: Genetically engineered mouse model

H&E: Haematoxylin and Eosin

HGSOC: High grade serous ovarian carcinoma

HIF: Hypoxia-inducible factor

HPV: Human papillomavirus

IHC: Immunohistochemistry

LGSOC: Low-grade serous carcinoma

LMM: Linear Mixed-Effects Model

MDSC: Myeloid-derived suppressor cell

MHC (I/II): Major histocompatibility complex

MMRd: Mismatch repair deficient

MS: Mass spectrometry

MSC: Mesenchymal stem cell

NCI: National Cancer Institute

NETs: Neutrophil extracellular traps

NGS: Next generation sequencing

NK: Natural Killer (cells)

NP: Nanoparticle

NSMP: No specific molecular profile

OC: Ovarian carcinoma

OS: Overall survival

OTSC: Organotypic tumour slice culture

p53abn: p53 abnormal

PCOS: Polycystic ovarian syndrome

PD: Parkinson's disease

PD-1 / PD-L1: Programmed cell death protein 1 / ligand 1

PDX: Patient-derived xenograft

PEG: Polyethylene glycol

PFS: Progression free survival

PLGA: Poly(lactic-co-glycolic acid)

POLEmut: POLE ultramutated

PVA: Polyvinyl alcohol

ROS: Reactive oxygen species

RT-qPCR: Quantitative reverse transcription polymerase chain reaction

SDS-PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis

TAA: Tumour associated antigens

TAM: Tumour associated macrophage

TAN: Tumour associated neutrophil

TCGA: The Cancer Genome Atlas

TILs: Tumour infiltrating lymphocytes

TLDA: TaqMan Low Density Array

TLS: Tertiary lymphoid structures

TME: Tumour microenvironment

Treg: Regulatory T cell

VEGF: Vascular endothelial growth factor

Chapter 1

Introduction

Section 1.1

Overview of Gynaecological Cancers and the Current Therapeutic Landscape

1.1.1 Gynaecological Cancers: An Overview

Gynaecological cancers are a broad group of female reproductive malignancies pertaining to the cervix uteri, corpus uteri (endometrium), ovary, vulva, vagina, and very rarely, the fallopian tube (Figure 1) (Zhu et al. 2024). The combined incidences of gynaecological cancers totalled ~1.4 million cases in 2020, making it the 2nd leading cause of cancer in women (Table 1) (WHO 2020; <https://gco.iarc.fr/today>). Globally, the incidence of gynaecological cancers has been rising with ageing populations and associated with genetic, dietary and environmental factors (Zhu et al. 2024). Due to the implementation of cervical screening and human papillomavirus (HPV) vaccination programmes, cervical cancer has reached stable incidence rates in many high-income countries, however ovarian and endometrial cancers remain on the rise, including in Western Europe and USA (Funston et al. 2018; Davies-Oliveira et al. 2021; Gilham and Peto 2025). Ovarian and endometrial cancers often have worse outcomes than cervical cancer due to symptoms appearing at later stages, when it is more aggressive, metastatic and harder to treat. Additionally, there are currently no screening or vaccination programmes for these malignancies (Funston et al. 2018).

Table 1. Gynaecological Cancer Incidence, Mortality and 5-year prevalence rates and rankings in women in the year 2020.

Cancer	Incidences (Ranking)	Mortalities (Ranking)	5-year prevalence (Ranking)
Cervix Uteri	604,127 (4 th)	341,831 (4 th)	1,495,211 (4 th)
Corpus Uteri/ Endometrium	417,367 (6 th)	97,370 (13 th)	1,415,213 (5 th)
Ovary	313,959 (8 th)	207,252 (8 th)	823,315 (7 th)
Vulva	45,240 (21 st)	17,427 (22 nd)	135,892 (20 th)
Vagina	17,908 (27 th)	7,995 (27 th)	44,613 (27 th)
Total	1,398,601 (2nd)	671,875 (2nd)	3,914,244 (2nd)

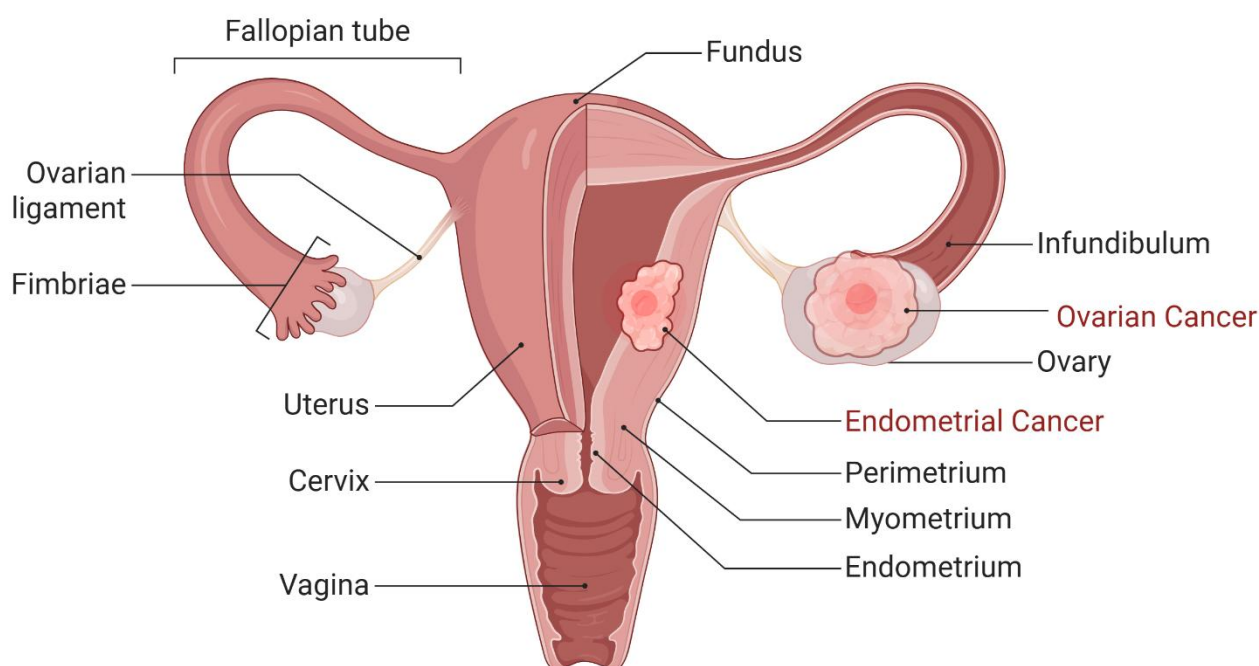


Figure 1. Female reproductive system and the development of ovarian and endometrial cancers. Made in Biorender.

1.1.2 Ovarian Cancer

Ovarian cancer is the 8th most prevalent malignancy in women and considered one of the most lethal gynaecological cancers (Figure 2) (Cancer Today, 2022; Tavares et al., 2024). Ovarian cancer can arise from transformation events occurring in the sex-cord stromal, germ, or epithelial cells, however most cases (90-95%) occur in epithelial cells, termed epithelial ovarian cancer (EOC) (Table 2) (Wei et al., 2025; Tavares et al., 2024). The risk of developing ovarian cancer increases with old age, with 75% of EOC cases occurring in women over 55 years old, as well as being linked to obesity, smoking, long-term use of hormone replacement therapies, and inherited genetic mutations in the homologous repair genes *BRCA1/2*. Whereas decreased risk has been associated with childbirth, oral contraceptives, and breast feeding (Funston et al. 2018). Symptoms are often absent until advanced stages, and include pelvic pain, bloating, urinary urgency, development of an abdominal mass, gain or loss of appetite and/or weight, and unexplained bowel habits. Despite extensive research into biomarkers that could be used as an ovarian

cancer screening test, such as CA125, current evidence does not show that population-based screening improves clinical outcomes, and therefore it is not currently recommended (Charkhchi *et al.*, 2020).

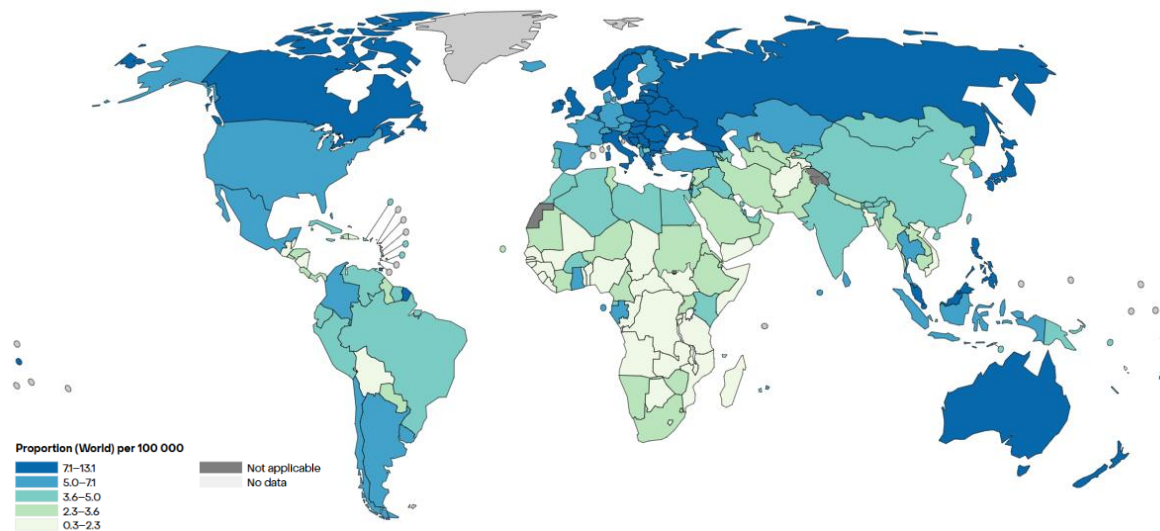


Figure 2. Prevalence of Ovarian Cancer in 2022. Estimated number of prevalent cases (1-year), as a proportion (World) per 100 000. From *Cancer Today*, GLOBOCAN (2022).

There are five main histological subtypes of EOCs categorised by their architecture and morphology: mucinous, clear-cell, endometrioid and serous (Table 2). The latter is further classified into high grade serous ovarian carcinoma (HGSOC) and low-grade serous carcinoma (LGSOC) based on the degree of cytological alterations (Kurman and Shih, 2016; Suster and Virant-Klun, 2019). As shown in Table 2, the most prevalent subtype of ovarian cancer is HGSOC accounting for 70% of all cases, and which nearly all cases (96%) are characterised by gene mutations in the tumour suppressor gene *TP53*, with ~13% also carrying alterations in *BRCA1/2* genes (Bell *et al.*, 2011; Suster and Virant-Klun, 2019).

HGSOC is considered one of the most devastating subtypes due to its association with an advanced-stage diagnosis, aggressive and metastatic phenotype, treatment-resistance, and poor 5-year survival rate of 30% (Satora *et al.*, 2024). Treatment plans for ovarian cancer typically follow a generic strategy of primary surgical debulking surgery

and secondary platinum-based chemotherapy. Whilst this therapeutic protocol may initially display efficacy in patients to the point of remission, approximately 75% of cases will ultimately recur within the first two years – many in the initial 6 months – by means of inherent or developed drug resistance (Cabarca *et al.*, 2024).

Table 2. Epithelial ovarian cancer subtypes including their percentage of occurrence, origin and associated genetic mutations

Epithelial Ovarian Cancer	% All EOC	Origin	Associated Genetic Mutations	Reference
High-Grade Serous Ovarian Carcinoma (HGSOC)	70%	Fallopian tube, ovary or peritoneum	<i>TP53</i> (96%) and <i>BRCA1/2</i> (13-20%), <i>PIK3CA</i> (18%)	Bell <i>et al.</i> , 2011; Barnes <i>et al.</i> , 2021
Endometrioid Ovarian Cancer (EnoC)	10%	Endometrium	<i>CTNNB1</i> (43%), <i>PI3KA</i> (43%), <i>ARID1A</i> (36%), <i>PTEN</i> (29%)	Hollis <i>et al.</i> , 2020; Barnes <i>et al.</i> , 2021
Ovarian Clear Cell Carcinoma (OCCC)	10%	Endometrium	<i>PIK3CA</i> (50%), <i>ARID1A</i> (45%), <i>TP53</i> (20%), <i>KRAS</i> (10%)	Barnes <i>et al.</i> , 2021
Low-Grade Serous Ovarian Carcinoma (LGSOC)	<5%	Fallopian tube	<i>KRAS</i> (20-35%), <i>BRAF</i> (10-40%), and <i>NRAS</i> (10-24%), <i>ERBB2</i> (5%)	Cheasley <i>et al.</i> , 2020; Barnes <i>et al.</i> , 2021
Mucinous Carcinoma	3%	Germ cells, transitional cells, endometrium	<i>KRAS</i> (54%), <i>PIK3CA</i> (8%), and <i>BRAF</i> (2%)	Panyavaranant <i>et al.</i> , 2019

1.1.3 Further Classification of HGSOC

It is well known that cancer, even that of the same subtype, is extremely heterogenous – and HGSOC is no exception. Numerous groups have applied multi-omic approaches in HGSOC patient samples to identify and characterise the multitude of cell populations and their molecular signatures within this disease subset, consequently allowing the further classification of HGSOC (Davidson *et al.*, 2023). One pioneering example of this

is by The Cancer Genome Atlas research network who through the exploration of HGSOC using next generation sequencing (NGS) and microarray analyses classified four distinct transcriptional signatures into the following subtypes: mesenchymal, proliferative immunoreactive, and differentiated HGSOC (Bell *et al.*, 2011). These defined subtypes have been useful in further predicting prognoses (Table 3). For example, mesenchymal HGSOC is characterised by high levels of stromal (FAP), angiogenic (*ANGPTL1*, *ANGPTL2*) and mesenchymal stem cell markers and is associated with poorer prognoses (Khashaba *et al.*, 2022). Similarly, proliferative HGSOC has also been linked to poor patient outcomes and is defined by a decrease in ovarian tumour markers (MUC1 and MUC16) and an increase in proliferative markers. In contrast immunoreactive subtypes have increased immune activation such as high expression of T-cell chemokine ligands (CXCL10 and CXCL11) and their target receptor (CXCR3), and are deemed more treatment responsive. Whereas differentiated subtypes are associated with high expression of MUC11 and MUC16 and are associated with more moderate outcomes (Bell *et al.*, 2011; Davidson *et al.*, 2023). Due to the molecular heterogeneity of HGSOC and its association with clinical outcome, it is vital that all these subtypes are carefully represented in the laboratory during drug development.

Table 3. HGSOC subtypes, their prognoses and molecular characteristics.

HGSOC Subtypes	Prognosis	Characteristics
Immunoreactive	Good	CXCL10, CXCL10, CXCR3
Differentiated	Moderate	↑MUC1, MUC16, <i>SLPI</i>
Mesenchymal	Poor	↑ <i>HOX</i> , FAP, <i>ANGPTL1</i>
Proliferative	Poor	↓MUC1 and MUC16, ↑ <i>MCM2</i> and <i>PCNA</i>

1.1.4 Endometrial Cancer

The endometrium is a specialised mucosal layer that lines the uterine cavity. Structurally, it is composed of two distinct regions: 1) the *stratum functionalis*, which is hormone-sensitive and undergoes cyclic proliferation and subsequent shedding during the menstrual cycle, and 2) the underlying *stratum basalis*, a permanent stromal layer which is responsible for the regeneration of the *functionalis* post-menstruation (Yamaguchi *et al.*, 2021). This dynamic tissue is the site of uterine malignancies, broadly classified based on their cellular origin. Endometrial carcinoma (EC) arises from the glandular epithelial cells and accounts for approximately 90 % of all uterine cancers, while uterine sarcoma arises from the underlying mesenchymal connective and muscle tissue, and accounts for the rare, yet highly aggressive remaining cases (Tai *et al.*, 2023). Like with ovarian cancer, risk factors include older age (~75% are >55 years old), genetic conditions e.g., Lynch syndrome, hormonal abnormalities such as late menopause, and obesity, with >50% of patients being clinically overweight (Onstad *et al.*, 2016; Funston *et al.*, 2018). There are also no cancer screening programmes for EC. One key sign of EC is unexplained long-term (>12 months) postmenopausal bleeding, occurring in up to 90% of cases. Patients can also present with abnormal vaginal discharge, abdominal pain, increased blood glucose levels, anaemia and blood in the urine (Funston *et al.*, 2018).

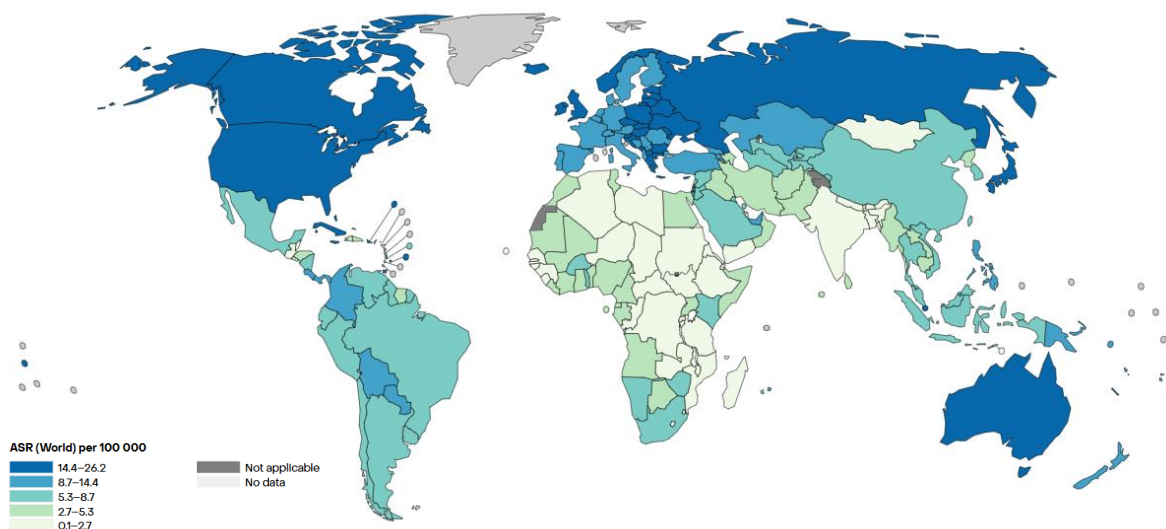


Figure 3. Prevalence of Corpus Uteri Cancer in 2022. Estimated number of prevalent cases (1-year), as a proportion (World) per 100 000. Data from Cancer Today, GLOBOCAN (2022).

EC is highly prevalent, ranking as the 6th most common cancer in women worldwide and resulted in 417,367 new diagnoses in 2020 (Figure 3) (Cancer Today, 2022). EC is further subclassified into distinct histological types: endometrioid, uterine serous carcinoma, and clear cell carcinoma (Table 4) (Hinshaw *et al.*, 2013). According to Bokhman classification (1993), endometrioid EC is a Type I, oestrogen-dependent subtype characterised by genetic perturbations in key signalling pathways, most notably *PTEN*, *PIK3CA*, and *KRAS* (Bokhman, 1983; Weigelt *et al.* 2013; Li *et al.* 2025). It is also the most prevalent EC subtype, comprising ~80% of all EC cases. While highly prevalent, endometrioid EC has a reasonably favourable prognosis when diagnosed early and treated with surgery and adjuvant chemotherapy, with an overall five-year survival of around 88% (Åkesson *et al.* 2023). However, 25% of patients are diagnosed in advanced stages, where the survival rate drastically drops to 18% for those with distal metastasis (Volinsky-Fremond *et al.* 2024; Tillmanns *et al.*, 2024; Brasseur *et al.*, 2016).

Table 4. Corpus Uteri Cancer subtypes including their percentage of occurrence, classification and associated genetic mutations

Uterine Corpus Cancer	Subtypes	% All Corpus Uteri Cancers	Bokhman Classification	Associated Genetic Mutations
Endometrial Carcinoma (90%)	Endometrioid	80%	Commonly Type I (oestrogen-dependent)	<i>PTEN</i> , <i>PIK3CA</i> , <i>KRAS</i>
	Uterine Papillary Serous Carcinoma	10%	Type II (Poor prognosis, oestrogen-independent)	<i>P53</i> , <i>HER2</i> , <i>P16</i>
	Clear Cell Endometrial Carcinoma	5%		
Uterine Sarcoma (10%)	Leiomyosarcoma	1% (70% of uterine sarcomas)		
	Endometrial Stromal Sarcoma	n/a (<1%)		
	Undifferentiated Uterine Sarcoma	n/a (<1%)		

1.1.5 Further Classification of Endometrial Cancer

Like with HGOSC, endometrial cancer was further classified into four distinct molecular subtypes based on TCGA research (Stelloo et al. 2016; Piulats et al. 2017). This research was used as the basis for the development of the new clinically applicable classification system: Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) and Leiden/TransPORTEC classification (Walsh et al. 2023). ProMisE methodology began being implemented for the therapeutic management of EC in 2021 following a published consensus by the European Societies of Gynaecological Oncology, Radiotherapy, and Pathology (Andrade et al. 2024).

The four molecular subtypes are *POLE* ultramutated (*POLE*mut), mismatch repair deficient (MMRd), p53 abnormal (p53abn), and no specific molecular profile (NSMP) (Table 5). In one study, MMRd, p53abn and NSMP had very similar prevalences, ranging from 27.2-28.9% in the study population, whereas *POLE*mut was slightly less frequent occurring in 15.8% (Andrade et al. 2024; Secord et al. 2025).

*POLE*mut tumours are characterised by mutations in the exonuclease domain of the *POLE* gene, responsible for encoding the DNA polymerase epsilon catalytic subunit, and consequently results in highly mutated tumours with numerous C→A nucleotide transversions due to poor DNA synthesis. However, these tumours have the most favourable prognosis of the four molecular subtypes, with almost no recurrence and extremely low disease-specific mortality rates (<1%) (Murali et al. 2014; Walsh et al. 2023). The MMRd subtype is characterised by high microsatellite instability (MSI-H) and hypermutated phenotype due to defective DNA mismatch repair. Approximately 10% of MMRd EC cases overlap with a Lynch syndrome diagnosis due to germline MMR gene mutations, whereas the remaining 90% are from somatic defects, primarily from hypermethylation of the *MLH1* promoter. Prognosis of these tumours is moderate and dependent on stage and degree of cytological alterations (Crosbie et al. 2022). In contrast, p53abn tumours are denoted by TP53 mutations, extensive gene copy-number alterations and low mutation rates. This group corresponds to the TCGA "copy-number high" or "serous-like" category and includes most serous carcinomas and some high-grade endometrioid carcinomas (Table 5). These tumours have the worst prognosis among the molecular subtypes (Murali et al. 2014; Walsh et al. 2023).

Lastly, NSMP comprises tumours devoid of POLE mutations and with wildtype MMR and p53. This subtype consists primarily of microsatellite-stable, low-grade endometrioid carcinomas. The NSMP category is further stratified by oestrogen receptor (ER) status, with ER-positive tumours having intermediate prognosis and ER-negative tumours having poorer outcomes similar to p53abn tumours (Walsh et al. 2023; Secord et al. 2025).

Table 5. Endometrial cancer subtypes (ProMisE Classification, 2021)

Endometrial Cancer Subtypes	Prognosis	Characteristics
<i>POLE</i> Ultramutated (<i>POLE</i> mut)	Good	<i>POLE</i> mutations, numerous C→A nucleotide transversions
Mismatch Repair Deficient (MMRd)	Moderate	High microsatellite instability, MLH1 promoter hypermethylation
p53 Abnormal (p53abn)	Poor	TP53 mutations, serous-like carcinomas
No Specific Molecular Profile (NSMP)	Moderate – Poor	ER-positive or ER-negative, wildtype: <i>POLE</i> , MMR and p53

1.1.6 Standard-of-Care and Targeted Therapy

Both in ovarian and endometrial carcinomas the standard-of-care is typically cytoreductive surgery with neoadjuvant chemotherapy using paclitaxel and carboplatin, administered intravenously on a 21-day cycle for 3 and 1 hours, respectively. In high-risk cases, radiotherapy is given in combination or sequentially (Azevedo et al. 2018). The last decade has also seen the emergence of targeted therapeutics, designed to manipulate cancer-specific deficiencies or hyperactivities. For example, in ovarian cancer, the FDA has approved the use of PARP inhibitors, monoclonal antibodies against angiogenic factors (Bevacizumab), and recently the folate receptor-targeting antibody drug conjugate ELAHERE (Mirvetuximab soravtansine-gynx) (Cortez et al., 2017; Satora et al., 2024). However, despite therapeutic advancements, only 13 distinct FDA-approved drug entities exist for ovarian cancer and more than 70% of patients will experience disease recurrence following treatment (Table 6) (Zhou et al., 2016; Cortez et al., 2017; Radu et al., 2023; Wojtyła et al., 2023; Tavares et al., 2024). In EC, the targeted landscape is also rapidly evolving, with recent FDA-approved options comprising checkpoint inhibitors (e.g., Dostarlimab-gxly, Durvalumab, Pembrolizumab), kinase inhibitors (Lenvatinib

mesylate), and hormone therapies (Megestrol Acetate) (Table 7) (Francoeur et al. 2025). Yet treatment recurrence occurs in 20% of patients, often presenting with treatment resistant, metastatic and incurable lesions, supporting the need for new therapeutic options.

1.1.7 Drug Discovery and Drug Repurposing

While there is a severely unmet need for new clinically approved therapeutics which are able to overcome drug resistance and extend survival rates, the research community are battling high drug development costs, timeframes, and attrition rates, with approximately 95% of new anti-cancer agents failing at clinical phase I trials (Prasad et al., 2017; He and Deng 2022). Therefore, recent efforts have been trying to optimise the drug discovery process by using advanced 3D laboratory models which can more realistically replicate the pathophysiology of tumours and more accurately predict clinical outcomes.

Moreover, drug repurposing has become a very attractive solution to overcome high drug discovery costs and lengthy timelines. Drug repurposing in oncology represents a strategy to identify anticancer activity in existing medications with established safety profiles, offering a faster and more cost-effective alternative to traditional drug development (Dhanush and Ganesh 2025). This approach leverages drugs already approved for other indications, reducing development timelines from 10-17 years to potentially much shorter periods and lowering costs significantly (Mohi-ud-din et al. 2023). Since the pharmacological profiles, side effects, and toxicity levels of these drugs are already well understood, the risk of unexpected adverse effects is lower. Drug repurposing can also provide new treatment options for cancers that lack effective therapies or have developed resistance to standard treatments (Mohi-ud-din et al. 2023; Dhanush and Ganesh 2025). Additionally, it encourages the exploration of novel mechanisms of action and may lead to the discovery of combinations that improve patient outcomes. Overall, drug repurposing could be a practical and efficient strategy in cancer therapy development.

Table 6. FDA Approved Medicines for Ovarian Cancer

<i>Drug</i>	<i>Common Names</i>	<i>Mechanism</i>	<i>Category</i>	<i>Reference</i>	<i>Year</i>
<i>Bevacizumab</i>	Alymsys, Avastin, Zirabev, Mvasi	VEGF-A blocker (anti-angiogenesis)	Monoclonal Antibody	McClung and Wenham, 2016	2014
<i>Carboplatin</i>	Paraplatin	DNA Damage	Platinum-based Chemotherapeutic	Monneret, 2011	1989
<i>Cisplatin</i>	Platinol	DNA Damage	Platinum-based Chemotherapeutic	Fernandez-Garza et al., 2021	1978
<i>Doxorubicin Hydrochloride</i>	Adriamycin	Topoisomerase II blocker	Anthracycline Chemotherapeutic	Fernandez-Garza et al., 2021	1995
<i>Gemcitabine Hydrochloride</i>	Gemzar, Infugem	Nucleoside Analog	Antimetabolite Chemotherapeutic	AbuShahin et al. 2007	2006
<i>Melphalan</i>	Alkeran	DNA and RNA Synthesis Inhibition	Alkylating Chemotherapeutic	Fernandez-Garza et al., 2021	2001
<i>Mirvetuximab soravtansine-gynx</i>	Elahere,	Folate receptor- α and microtubule inhibition	Monoclonal Antibody/ anti-microtubule chemotherapy	FDA.gov, 2024	2024
<i>Niraparib</i>	Zejula	PARP Inhibition	Small Molecule Inhibitor (SMI)	Fernandez-Garza et al., 2021	2017
<i>Olaparib</i>	Lynparza	PARP Inhibition	SMI	Arora et al. 2021	2014
<i>Paclitaxel</i>	Taxol	Microtubule Inhibition	Anti-microtubule Chemotherapy	Khanna et al. 2015	1992
<i>Rucaparib (Camsylate)</i>	Rubraca	PARP Inhibition	SMI	Balasubramaniam et al. 2017	2016
<i>Thiotepa</i>	Tepadina	DNA and RNA Synthesis Inhibition	Alkylating Chemotherapeutic	Fernandez-Garza et al., 2021	1959
<i>Topotecan Hydrochloride</i>	Hycamtin	Topoisomerase II blocker	Chemotherapeutic	Fernandez-Garza et al., 2021	1998

Table 7. FDA Approved Medicines for Endometrial Cancer

<i>Drug</i>	<i>Common Names</i>	<i>Mechanism</i>	<i>Category</i>	<i>Year</i>
<i>Carboplatin</i>	Paraplatin	DNA Damage	Platinum-based chemotherapeutic	-
<i>Dostarlimab-gxly</i>	Jemperli	Anti-PD-1 Inhibitor	Monoclonal Antibody	2024
<i>Durvalumab</i>	Imfinzi	Anti-PD-1 Inhibitor	Monoclonal Antibody	2024
<i>Lenvatinib mesylate</i>	Lenvima	Tyrosine kinase inhibitor	Anti-angiogenic	2021
<i>Megestrol Acetate</i>	Megace	Synthetic Progesterone	Hormone Therapy	1971
<i>Paclitaxel</i>	Taxol	DNA Damage	Taxane chemotherapeutic	-
<i>Pembrolizumab</i>	Keytruda	Anti-PD-1 Inhibitor	Monoclonal Antibody	2024
<i>Trastuzumab deruxtecan</i>	Enhertu	Anti-HER2 ADC	Antibody Drug Conjugate	2024

Section 1.2

The Tumour Microenvironment and Therapeutic Resistance

Our knowledge of the architecture and pathophysiology of a solid tumour has expanded rapidly over the last few decades; it is now well accepted that a tumour is not simply a clump of mutated cells growing in an uncontrolled state, but a complex, 'organ-like' mass composed of a multitude of heterogeneous cell networks with an intricate three-dimensional (3D) architecture and numerous interactions and signalling cues that promote tumour growth and therapeutic evasion. This complex ecosystem is typically referred to in two parts: the rapidly dividing tumour bulk and the tumour microenvironment (TME). The TME refers to the space which surrounds and supports the tumour bulk, and is composed of the:

- 1) Vasculature providing oxygen and nutrient transport (but leaky enough to interfere with drug delivery),
- 2) Stroma, rich in cancer associated fibroblasts (CAFs), adipocytes, and mesenchymal stem cells (MSCs) which control extracellular matrix (ECM) production, density, and stiffness, impacting both immune cell migration and drug penetration.
- 3) Immunological landscape comprised of dysregulated immune cells involved in immune shielding and cancer evasion,
- 4) Cancer stem cells (CSC), a subpopulation of cancer cells which possess metastatic, self-renewal, treatment-evading, and multilineage differentiation properties, and therefore can reinstate tumorigenesis following therapy,
- 5) Infiltrated nerve system linked to cellular sensing, inflammation, angiogenesis and metastatic processes.

The interaction between tumour cells and their microenvironment significantly influences tumour progression and treatment response in gynaecological malignancies (Figure 4). In these cancers, most notably (HGSOC), the TME harnesses resistance to chemotherapy, targeted agents, and immunotherapy through adaptive signalling, metabolic rewiring, and physical and immunological barriers. These mechanisms enable tumour survival despite therapeutic interventions, resulting in recurrence and poor clinical outcomes (Zhao et al. 2012; Liu et al. 2021b; Garlisi et al. 2024). In this section, I will discuss the different elements of the TME and how they contribute to the generation of a pro-tumorigenic niche with therapeutic resistance.

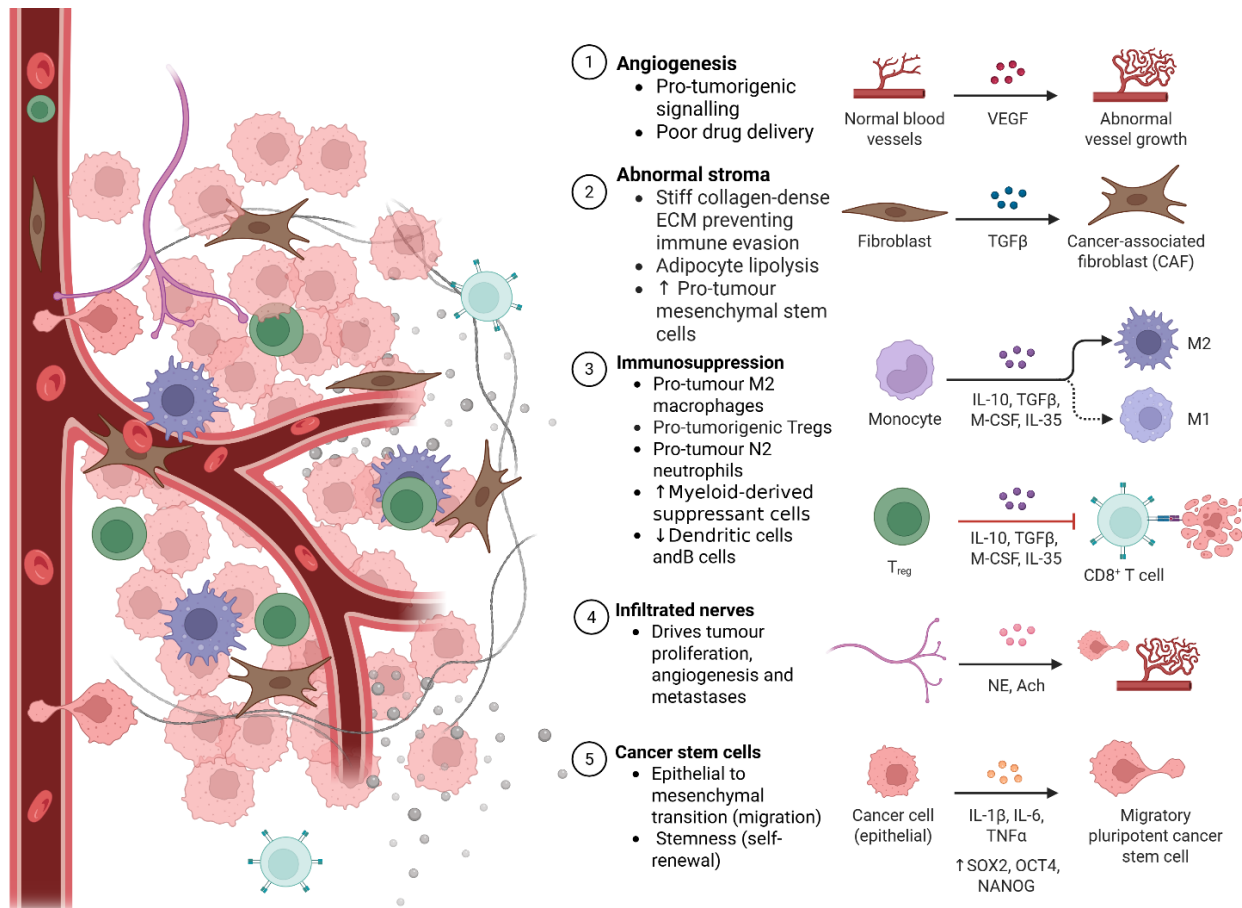


Figure 4. The tumour microenvironment in gynaecological cancers. Five key changes occur to create a pro-tumorigenic niche: 1) angiogenesis where new blood vessels sprout to support tumour growth, 2) abnormal stroma characterised by activated cancer associated fibroblasts (CAFs) and a stiff collagen-dense matrix, 3) immunosuppressive immune cells such as CD4⁺ Treg cells and anti-inflammatory M2 macrophages, 4) infiltrated nerves that drive cancer cell proliferation, metastases and angiogenesis, and 5) cancer stem cells with metastatic and self-renewal potential. Made in Biorender.

1.2.1 Tumour Vasculature

Angiogenesis is the mechanism by which new blood vessels sprout from the preexisting vasculature, expanding oxygen and nutrient delivery and waste removal to new regions of the tissue. Without an adequate perfusion system, the tumour's high metabolic demands become the limiting factor in its expansion, preventing the mass from growing beyond 1-

2 mm³. Hence, a proangiogenic environment and the establishment of a large vascular network are crucial for a tumour's growth and survival. Consequently, this process often becomes dysregulated within a solid tumour (Figure 4) (Matuszewska et al. 2021).

As the tumour grows, the inner most region starts to become hypoxic, stimulating the release of hypoxia-inducible factors (HIFs), such as HIF-1 α , and reactive oxygen species (ROS) from tumour cells. These mediators trigger the 'angiogenic switch' – a functional shift characterised by the upregulation of pro-angiogenic factors, including vascular endothelial growth factor A (VEGF-A), alongside the suppression of anti-angiogenic mediators like angiostatin and thrombospondin. In ovarian cancer, high serum levels of VEGF correlate with poorer progression free survival and overall survival (Yu *et al.*, 2013). As a counteractive measure to this tumour idiosyncrasy, the VEGF-neutralizing monoclonal antibody, Bevacizumab, has been approved for ovarian cancer, improving progression free survival (PFS) when administered as a second line therapy (Erciyestepe *et al.*, 2025).

Similarly in endometrial cancer, angiogenesis also plays a critical role in endometrial cancer progression with high VEGF expression seen in 56% of endometrial cancers and is an independent predictor of shorter disease-specific survival (Kamat et al. 2007). However, benefits of Bevacizumab show varied responses with some clinical trials demonstrating no significant differences in PFS as a monotherapy and in combination with chemotherapy (Simpkins et al. 2015; Lorusso et al. 2019; Rubinstein et al. 2021)

The vasculature within tumours also has an atypical morphology, characterised by a large network of small, disorganised leaky vessels that creates a higher interstitial pressure to the tumour core. This pressure gradient opposes immune infiltration and an inward flow of drugs, impairing the adequate delivery of pharmaceuticals (Stylianopoulos et al. 2018). Altogether, these studies highlight the critical role of the vasculature in tumour growth, survival, and drug delivery and thus, demonstrate the importance of its inclusion in preclinical models during anti-cancer drug discovery.

1.2.2 Stroma and Extracellular Matrix

The stroma is home to a number of non-malignant cell types involved in extracellular matrix production and remodelling, including fibroblasts, adipocytes, and mesenchymal stem cells. These cells secrete collagen, laminin, fibronectin and a diverse supply of biochemical signals which collectively control the structure, density, and stiffness of the ECM (Mun et al. 2024). These processes can also become dysregulated in cancer.

(a) CAFs

CAFs are one of the most prominent cell types of the TME and involved in important pro-tumorigenic processes such as fibrosis, inflammatory signalling, immune exclusion and ECM remodelling (Figure 4) (Zhang et al. 2022a).

In ovarian cancer, four subtypes of CAFs have been identified: CAF-S1, -S2, -S3, and -S4. These subtypes differ in their expression of biomarkers, including fibroblast activation protein (FAP), α -smooth muscle actin (α -SMA) and integrin β 1 (CD29), and their association with cancer subtype and prognosis. For instance, CAF-S1 (FAP^{high} CD29^{med-high} SMA^{high}) are associated with mesenchymal HGSOC and poor patient outcomes (Zhang et al. 2022a). CAFs have been shown to excessively produce stiff, dense regions of collagen that prevent immune cell migration and drug penetration. Moreover, they can also overproduce matrix metalloproteinases (MMPs) involved in matrix remodelling during tumour cell invasion, as well signalling factors e.g., VEGF, epidermal growth factor (EGF) and hepatocyte growth factor (HGF), that initiate tumour proliferation (Davidson et al. 2014; Santi et al. 2018; Axemaker et al. 2024). In mesenchymal HGSOC, tumours exhibit high levels of stromal components, increased ECM stiffness and are associated with metastasis and chemoresistance (Mieulet *et al.*, 2021).

Similarly, in endometrial cancer, CAFs also secrete higher levels of growth factors and cytokines compared to normal fibroblasts, including IL-6, IL-10, VEGF, EGF, and FGF (Subramaniam et al. 2013; Wang et al. 2018). These secreted factors activate key signalling pathways in cancer cells, particularly the PI3K/Akt and MAPK/Erk pathways, which drive angiogenesis, proliferation and survival (Subramaniam et al. 2013). Another

factor, Stromal Cell-Derived factor (SDF-1/CXCL12) has also been identified as a key molecule in endometrial cancer biology through binding and stimulating the CXCR4 receptor on endometrial cancer cells. Elevated levels of SDF-1 α in endometrial cancer patients correlate with advanced clinical features, such as deep invasion of the myometrium and metastasis to the lymph nodes, ultimately leading to lower survival rates. Interestingly, blocking the SDF-1/CXCR4 axis with a CXCR4 antagonist (AMD3100) significantly inhibited CAF-mediated tumour progression (Teng et al. 2016). Like in HGSOC, elevated ECM stiffness in endometrial cancer correlates with more aggressive tumour behaviour and reduced sensitivity to chemotherapy (Sun et al. 2025). Hence, CAFs are fundamental to tumour biology and drug sensitivity.

(b) Adipocytes

Adipocytes also play an interesting role in tumorigenesis through metabolic reprogramming. Cancer-associated adipocytes are irregularly-shaped dedifferentiated cells filled with small, dispersed lipid droplets that are undergoing dysregulated lipolysis within the TME (Attané and Muller 2020; Cho et al. 2023). This excessive lipolysis is stimulated by cancer cells and leads to free fatty acid secretions from adipocytes, which are subsequently used by cancer cells as a metabolic fuel that drives tumour growth (Attané and Muller 2020). These lipolytic adipocytes are frequently found near aggressive tumours. In fact, adipocyte rich regions, such as the adipose tissue of the omentum, are the preferred regions of gynaecological tumour metastases (Duarte Mendes et al. 2023). Moreover, direct adipocyte-tumour cell interactions can trigger metastasis and chemoresistance in HGSOC through inducing lipid chaperone (*FABP4*) expression (Mukherjee et al. 2020). Beyond their role in metabolic rewiring and metastasis, adipocytes can also directly promote tumour proliferation and survival, for example, through releasing pro-tumorigenic adipokines like leptin and reducing the production of anti-tumour adiponectin. High leptin and low adiponectin levels are associated with obesity and are also independently associated with increased risks of developing ovarian and endometrial cancers (Ray et al. 2018; Tsankof and Tziomalos 2022). Hence, inclusion of adipose tissue is essential for studying tumour initiation, metastasis and chemoresistance.

(c) Mesenchymal Stem Cells

Cancer-associated mesenchymal stem cells (MSCs) are non-malignant stromal progenitor cells with multipotent potential that differentiate into the mesodermal lineages, such as those of the bone (osteoblasts), cartilage (chondrocytes), fats (adipocytes), muscle (myocytes), and ECM-producers (fibroblasts). They also possess the ability to generate cell types of other lineages including endothelial cells, neuronal-like cells and cardiomyocytes (Atiya et al. 2020). While they are considered important players in wound healing, they have also been implicated in driving the development of tumorigenesis by giving rise to dysregulated cancer-associated adipocytes and fibroblasts, and by inducing an epithelial-mesenchymal transition (EMT) within cancer cells (Coffman et al. 2018). This EMT shift increases the ‘stemness’ of cancer cells, where they acquire a more aggressive, proliferative, and metastatic phenotype (Atiya et al. 2023).

In HGSOC, samples with increased MSCs and an EMT phenotype were more immunosuppressive, invasive and migratory, and are associated with poorer outcomes (Chirshev et al. 2020; Hu et al. 2020; Zhang et al. 2024). In endometrial cancer tissue samples, MSCs contribute to 1-5% of the total population and are said to have a dualistic role, in which they can be both anti-tumour and immunosuppressive (Wang et al. 2022). For instance, endometrium-derived MSCs can suppress endometrial cancer cell viability, proliferation, and xenograft tumour growth more effectively than adipose-derived or umbilical cord-derived MSCs (Xu et al. 2023). The proposed mechanism involves secretion of Dickkopf-related protein 1 (DKK1), which inhibits Wnt/ β -catenin signalling in cancer cells, thereby suppressing cancer cell stemness and proliferation (Xu et al. 2023). In contrast, other endometrium-derived MSCs can create an immunosuppressive TME through upregulation of the programmed cell death-1 (PD-1/PD-L1) axis, consequently reducing the cytotoxicity of immunotherapies (Wang et al. 2022).

1.2.3 Tumour Immunological Landscape

The stroma is also the region in which tumour infiltrating immune cells can be found. Ovarian cancer is typically considered a ‘cold’ tumour characterised by a significant lack of effector T lymphocytes or by the presence of these cells in an immunosuppressed or ‘exhausted’ state (Garlisi et al. 2024). Other key indicators of low tumour immune response include an increase in intrinsic immune response blockades e.g., PD-1/PD-L1 engagement, impaired antigen presentation to T cells, and an enrichment of immunosuppressive immune cells, such as tumour associated macrophages (TAM), T regulator cells (Treg) and myeloid-derived suppressor cells (MDSC) (Figure 5) (Garlisi et al. 2024; Wang et al. 2024). The immunological landscape also varies drastically depending on the subtype, for example all molecular subtypes of HGSOC (except immunoreactive HGSOC) lack high levels of immune activation (Khashaba et al. 2022). Consequently, many attempts to utilise the immune system as anti-cancer strategy in HGSOC have performed poorly in clinical trials, such as CAR-T therapy and anti-PD-L1 monoclonal antibody blockades (Yang et al. 2022)

Similarly, in endometrial cancer the four molecular subtypes also have distinct immunological profiles. For instance, POLE-mutated and MMRd tumours are characterised by high immune infiltration and immune activation, with a particularly high abundance of CD8+ tumour-infiltrating lymphocytes (Dai et al. 2022; Wang et al. 2023b). These tumours respond exceptionally well to immune checkpoint inhibitors (Zhao et al. 2025). In contrast, p53-aberrant tumours typically exhibit an immunosuppressed phenotype with the highest densities of PD-L1+ macrophages and PD-1+ T cells, suggesting immune exhaustion despite some immune infiltration (Dai et al. 2022; Ariyanayagam and Baksh 2024). Whereas the no specific molecular profile (NSMP) subtype displays an ‘immune-cold’ phenotype with low overall immune cell infiltration (Dai et al. 2022)

The balance between both anti-tumour and pro-tumour immune cells within the TME of HGSOC and EC strongly influences prognosis and therapy response (Figure 5). Hence, strategies which enhance both immune infiltration and activation are needed (Liu et al. 2021b; Dai et al. 2022; Khashaba et al. 2022). This highlights the importance of immune inclusion in drug discovery models, both for efficacy and mechanistic studies of novel

therapies. The next subsections will give an overview of the role of lymphoid and myeloid cells in HGSOE and EC tumour progression.

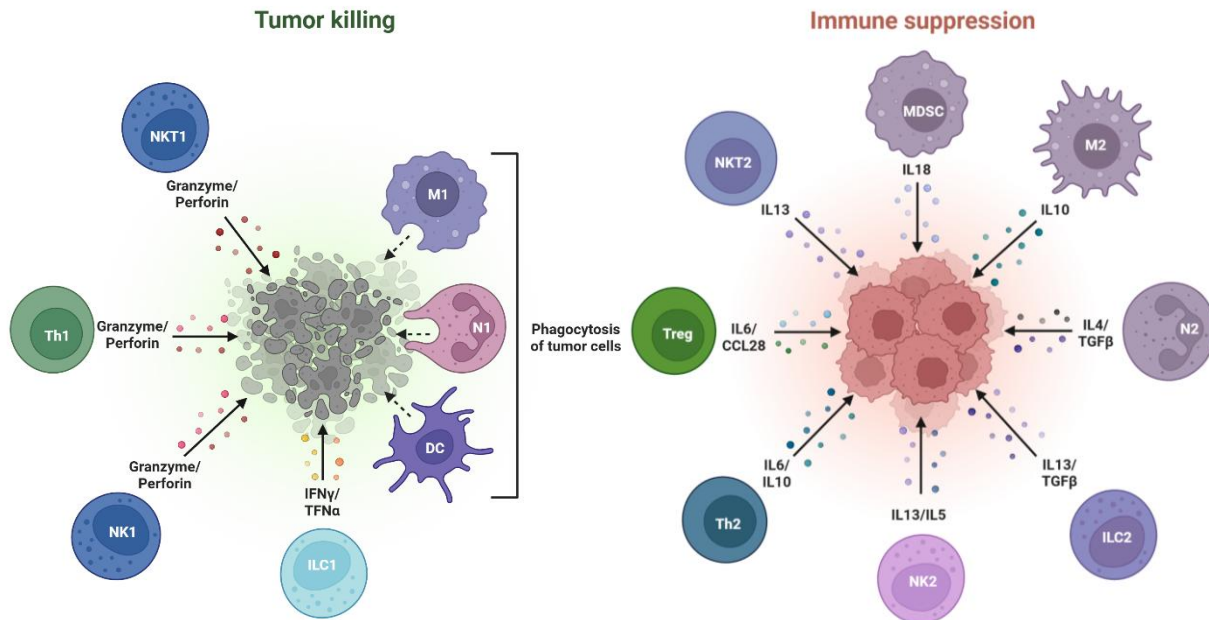


Figure 5. Immune activation and suppression in tumours. Tumour killing immune populations are characterized by abundant immune infiltration with increased CD8⁺ T cell and NK cell populations, M1 macrophages, N1 neutrophils and dendritic cells, making them more responsive to immunotherapies. Immune suppression in tumours exhibit scarce immune infiltration, primarily composed of regulatory T cells (Tregs), and low CD8⁺ T cell, natural killer (NK) cell and dendritic cell presence, often evading immunotherapies. Made with Biorender.

1.2.4 Tumour Infiltrating Lymphocytes (TILs)

TILs refer to immune cells derived from lymphoid progenitors such as CD4⁺ (helper) and CD8⁺ (cytotoxic) T cells, Natural Killer (NK) cells and B cells (Zhang et al. 2023; MacFawn et al. 2024). The infiltration and formation of functional and heterogenic immune hotspots of T and B cells, termed tertiary lymphoid structures (TLS), have been identified as clinical indicators of immunotherapeutic response and improved survival. However, their presence varies within the different morphological and molecular gynaecological

cancer subtypes and are closely associated with prognosis and therapeutic efficacy (Zhang et al. 2023). TLS are absent in HGSOC in approximately 85% of patients, and their absence has been linked to pro-tumorigenic stroma and ca-MSCs associations (MacFawn et al. 2024). Similarly, TLS are found in 19% of EC cases and are more abundant in POLEmut and MMRd subtypes (Horeweg et al. 2022a). However, the mechanism behind TLS suppression remains mostly unelucidated.

(a) CD4+ T cells

CD4+ T cells orchestrate the immune response upon recognition of antigens displayed by major histocompatibility complex II (MHC II) molecules on antigen presenting cells (APCs), i.e., dendritic cells, macrophages and B cells. Following recognition, CD4+ cells release cytokines such as IFN- γ and IL-2 that triggers the maturation of APCs and the activation and clonal expansion of CD8+ T cells (Kruse et al. 2023). However, the CD4+ lineage also includes a specialised immunosuppressive subset termed Regulatory T cells (Tregs), characterised by the expression of the transcription factor FOXP3 (Lalfer et al. 2019).

In HGSOC and endometrial cancers, CD4+/FOXP3+ cells are associated with an immunosuppressive pro-tumour phenotype and poor survival outcomes (Kolben et al. 2022; Mikulak et al. 2025). One study showed Tregs were virtually absent in healthy donor endometrium but positively associated with higher grades of endometrial cancer. Moreover, co-culture models of endometrial cancer and Treg cells increased cancer cell invasion, migration and survival (Kolben et al. 2022). Tregs are also particularly abundant in stage I HGSOC tumour lesions and positively correlate to worse prognosis (Mikulak et al. 2025). However, one study found that the precise location of Treg cells within the HGSOC TME alter their tumorigenic effect, for instance Treg cells in close proximity to metabolically inactive tumour cells correlated to worse survival outcomes, whereas Tregs that localised with highly metabolic and/or proliferating tumour cells resulted in more favourable prognoses (Berrell et al. 2025). This underpins the necessity for models which maintain the native TME cell-cell interactions and crosstalk when exploring tumour biology and drug efficacy.

(b) CD8⁺ T cells and NK Cells

In contrast, CD8⁺ T cells and NK cells act as the primary anti-tumour effectors by inducing apoptosis in malignant cells. High densities of CD8⁺ TILs generally predict more favourable clinical outcomes and improved overall survival in both HGSOC and EC. However, in HGSOC and EC, CD8⁺ T cells are often scarce or in a dysfunctional exhausted state, characterised by the expression of PD-1⁺ and TIM-3⁺ (Degos et al. 2019; Connor et al. 2024). These exhausted T cells have a distinctive phenotype characterised by low expression of cytokines, reduced cytotoxicity, and low clonal expansion, resulting in limited responses to chemotherapy and immunotherapy (Yang et al. 2022; Connor et al. 2024). Moreover, in endometrial cancer samples, tumour-resident NK cells also display an inhibited phenotype (Tigit⁺ and TIM-3⁺) and secrete less pro-inflammatory cytokines (IFN- γ , TNF- α and GrzB) than adjacent NK cells, suggesting the TME may be dampening the activity of these cells (Degos et al. 2019). Recently, bi-specific and tri-specific engager antibodies have been developed to activate exhausted T cells and NK cells within the gynaecological TME. These antibody modalities bind to specific cell surface proteins to unify effector cells and cancer cells and elicit dual-stimulation (O'Brien et al. 2025). For example, one tri-specific killer engager (TriKE) contained modalities towards CD16 on NK cells to drive activation, an IL-15 molecule to drive NK priming, expansion and survival, and HER2 on ovarian cancer cells, ensuring activated effectors came into close proximity with the malignant cells (Vallera et al. 2021). However, advanced *ex vivo* models that incorporate the TME, including heterogenic immune effector cells, are required to fully ascertain drug efficacy and mechanistic effects.

(c) B Cells

B cells are APCs responsible for antigen presentation to cytotoxic effectors to elicit an immune response. They are also capable of targeting malignant cells directly through their differentiation into plasma cells that secrete antibodies against tumour associated antigens (TAA), and memory B cells which maintain a long-lived memory response against the TAAs, rapidly reactivating upon re-exposure (Vledder et al. 2024; Yang et al.

2024). TIL B cells, particularly when organised within mature TLS, have been identified as an independent positive prognostic factor in gynaecological cancers (Horeweg et al. 2022; Kasikova et al. 2024). In HGSOC, B cell-infiltrated TLS were less common in primary tumours but more prevalent in HGSOC metastases. Moreover, B cell depletion was linked to immune checkpoint insensitive T cells (Kasikova et al. 2024). Similarly, in EC, patients with B-cell-rich TLS, especially those containing mature germinal centres, showed better CD8⁺ T cell function and progression-free survival compared to those without these structures (Horeweg et al. 2022). However, there are still major gaps in our understanding of why the majority of gynaecological cancer patients do not form mature TLS within their lesions.

1.2.5 Myeloid Compartment

(a) Tumour-associated Macrophages (TAMs)

TAMs are one of the most predominant immune cell types within the TME. They are described to have two different phenotypes: M1 (anti-tumour) and M2 (pro-tumour) (Figure 5). Within both HGSOC and EC, there is a clear polarisation of TAMs towards the immunosuppressed M2 state, characterised by increased M2 receptor expression (CD163, CD204, and CD206) and upregulated co-regulatory receptors: TIGIT, CD226, TIM-3, and LAG-3 (Brauneck et al. 2023). M2-TAMs are also shown to release the angiogenic driver VEGF and immunosuppressive cytokines, IL-10 and TGF- β , involved in inhibiting CD8⁺T cell and NK cell activity (Wang et al. 2025b). In contrast, M1 macrophages secrete pro-inflammatory cytokines e.g., TNF- α , IL-12, IL-1 β and promote antigen presentation to effector cells. The increased presence of M1 macrophages is significantly associated to long-term survival in HGSOC (Berry et al. 2021). Moreover, M1 macrophages were significantly more prevalent in samples of cured low-grade endometrioid carcinomas compared to those from recurrent patients, which presented almost no M1 macrophages and a small number of TAMs with an M2 phenotype (Nagamine et al. 2025). Hence, strategies that trigger M2 to M1 repolarisation are being explored as an immunotherapy strategy (Zhu et al. 2025).

(b) Myeloid-Derived Suppressor Cells

MDSCs are a heterogenic population of immature mature myeloid cells with immunosuppressive abilities that prevent the recruitment, priming and expansion of T cells. They are divided into three subsets: mononuclear-, polymorphonuclear- and early-stage- myeloid-derived suppressor cells (M-MDSC, PMN-MDSC, eMDSC). In ovarian cancer patients, M-MDSC produces arginase and ROS which suppress T cell proliferation. Moreover, their presence was significantly higher in the peripheral blood and ascites of ovarian cancer patients, compared with healthy individuals which have almost a complete absence of this cell type (Okta 2023). M-MDSC abundance also inversely correlated with OC relapse-free survival (Wu et al. 2017). In endometrioid carcinomas, MDSCs also display strong immunosuppressive activity, inhibiting CD8⁺ T cell activity and stimulating angiogenesis *in vitro* and *in vivo* (Mabuchi and Sasano 2021; Jones et al. 2023). Thus, their representation in drug discovery models is important when evaluating new therapies, especially those related to T cell activation or T-cell mediated cytotoxicity, such as CAR-T therapy.

(c) Dendritic Cells

Dendritic cells are antigen-presenting phagocytes derived from haematopoietic stem cells that play a key role in initiating and regulating the immune response in the TME (Lee et al. 2023). They do this by engulfing and presenting tumour-associated antigens on their cell surface via MHC I and II receptors to T cells (Vander Lugt et al. 2013; Gutiérrez-Martínez et al. 2015). There are two conventional DC subtypes: type 1 (cDC1) and type 2 (cDC2). cDC1 are the main cytotoxic CD8 T cell activators, whereas cDC2 produce IL-10 and TGF- β to stimulate T cell differentiation into the CD4 T helper cell subset (Mastelic-Gavillet et al. 2020; Mastelic-Gavillet et al. 2023). However, within the ovarian cancer TME, DCs are often scarce or in a phenotypically impaired state with an immunosuppressant function (Figure 5). In response to chemotherapy, ovarian cancer patients have lower frequencies of the cDC1 subtype compared to both pre-treatment levels and to healthy controls. Moreover, low cDC1 levels post treatment corresponds to poorer patient outcomes, suggesting this deficiency could be linked to chemoresistance

(Mastelic-Gavillet et al. 2023). In contrast, in endometrial cancer, dendritic cells are more abundant, with certain subsets (S100+, HLA-DR+) being associated with lower clinical stage and lymph node metastases (Zhao et al. 2012). Therefore, DC vaccination has been suggested as a potential strategy for DC activation alongside other therapeutic regimens (Lee et al. 2023).

(d) Tumour Associated Neutrophils (TANs)

Neutrophils are the most abundant leukocyte in the blood and are the first responders during infections and inflammation. Classically, mature neutrophils are considered a non-proliferative, short-lived (<1 day) and terminally-differentiated subset, however recent evidence suggests they are capable of survival up to 5.4 days with a greater level of plasticity and heterogeneity than once thought (Pillay et al. 2010; Hedrick and Malanchi 2021).

Neutrophils also play a part in tumour progression; Like with TAMs, TANs are classified into two populations: N1 (anti-tumour) and N2 (pro-tumour), whereby their phenotype is defined by molecular signals within the TME (Figure 5) (Cheng et al. 2025). In the presence of IFN- γ , N1 TANs are stimulated to secrete TNF-related apoptosis-inducing ligand (TRAIL) that induces apoptosis in cancer cells through the Death Receptors (DRs) (Cheng et al. 2025). However, it has been shown that both HGSOC and endometrial cancer patients have an N1/N2 polarisation, leading to an increase in N2 TAN-cancer cell interactions that promote metastases (Chen et al. 2024). In HGSOC, neutrophils demonstrated an immunosuppressant function on T cells, but not in non-tumour sites, suggesting the TME likely plays a role in TAN function (Emmanuelli et al. 2024). Interestingly, deletion of the stress sensor (IRE1 α) in TANs led to increased survival in the HGSOC *in vivo* models (Emmanuelli et al. 2024). In endometrial carcinomas, patients with lower ratios of neutrophils to lymphocytes also had significantly increased overall survival compared to patients with higher neutrophil-to-lymphocyte ratios (92.8 vs. 72.3 months) (Kirsch-Mangu et al. 2025).

Uncontrolled immune defence strategies, such as the extracellular release of decondensed DNA and granule enzymes termed Neutrophil Extracellular Traps (NETs)

are gathering more attention as a potential mechanism of chemoresistance in gynaecological cancers, particularly ovarian cancer (Chen et al. 2024). While NETs are typically released to entrap and kill microorganisms, within the TME they have been suggested to colocalise and inactivate chemotherapeutics (Tamura et al. 2022). Moreover, recent studies found multiple biomarkers of NETs were significantly higher in HGSOC patient plasma compared to healthy controls, and that NETs were also present in the TME (Kim et al. 2022; Tomás-Pérez et al. 2023).

1.2.6 Cancer Stem Cells

Cancer stem cells are a subpopulation of malignant cells with self-renewal and multi-differential potential, capable of reinstating tumorigenesis following therapy. They typically have a distinct phenotype of ‘stem-like’ markers such as CD133 (PROM1), CD24 (P-selectin), CD44 (hyaluronan receptor), ALDH1A1 (aldehyde dehydrogenase), CXCR4 (C-X-C motif chemokine receptor 4) and EPCAM (epithelial cell adhesion molecule), related to EMT behaviours, survival and metastasis (Cioffi et al. 2015; Iżycka et al. 2023). Moreover, CSC genetic signatures are characterised by increased expression of transcription factors NANOG, SOX2/9 and OCT4 related to a pluripotent state (Cioffi et al. 2015).

The clinical significance of these markers, however, appears to be highly context-dependent. In ovarian cancer, a subpopulation of CXCR4⁺/CD133⁺ cells have been identified as a driver of cisplatin resistance in both *in vivo* murine models and primary patient biopsies. Moreover, CD133⁺ cells were present in approximately 50% ovarian cancer samples and correlate with worse PFS and OS (Ruscito et al. 2017). In contrast, endometrial carcinomas demonstrate higher frequency of CD133⁺ cells (61%) and was associated with reduced vascular invasion and increased overall survival (Mancebo et al. 2017). This highlights the distinct nature of CSCs within different TME of gynaecological cancers and the need to further characterise CSC markers for outcome prediction.

1.2.7 Tumour Infiltrating Nerves

The peripheral nervous system is a vital neuronal network involved in environmental sensing, motor control and autonomic regulation of involuntary physiologic processes, including heart rate, blood pressure, and digestion (Wang et al. 2025). In addition, the autonomic nervous system (ANS) has also been shown to play a role in menstruation, particularly in ovarian blood flow, pain, and hormone cycles (Berga and Naftolin 2015; Yu et al. 2024). The ovaries and uterus are highly innervated by a dense network of autonomic and sensory nerve fibres closely associated with the vasculature. Autonomic nerve dysfunction has been linked to conditions such as polycystic ovarian syndrome (PCOS) and endometriosis, characterised by high levels of pain, hormonal imbalances, blood flow abnormalities and inflammation (Tong et al. 2020; Wei et al. 2020; Yu et al. 2024).

Increasing evidence indicates that the nervous system can also be a key orchestrator in tumorigenesis, angiogenesis, metastases and immune evasion. Tumour cells can migrate along neural pathways during metastatic spread, while nerve–tumour crosstalk, mediated by neurotrophic factors (e.g. NGF, BDNF) and neurotransmitters, promotes both nerve infiltration into the TME and enhanced tumour growth and invasion. In addition, neurotransmitters such as norepinephrine have been shown to attract immunosuppressive TAMs, MDSCs and Tregs that inhibit T cell and NK cell defences (Makale et al. 2017; Bayraktar et al. 2024; Yu et al. 2025).

Recent advances in intra-tumoral nerve-tracing have clarified this relationship in HGSOC, revealing a distinct shift in tumour innervation during malignancy, whereby predominantly sympathetic innervation in healthy ovarian tissue is replaced by the selective recruitment of sensory axons within the tumour microenvironment (Barr et al. 2021). Similarly, in endometrial cancer, cancer cells exploit the organ's intrinsic neuroplasticity, using neurotransmitters like norepinephrine and acetylcholine to promote epithelial proliferation and migration (Español et al. 2022). The relationship between endometrial cancer and the nervous system is further underscored by rare but aggressive endometrial metastases into the central nervous system (Ross et al. 2025). However, there are still significant gaps in our knowledge of how the nervous system is used by tumours to metastasise and evade therapeutic strategies.

Section 1.3

Preclinical Models for Drug Discovery in Gynaecological Cancer and Their Limitations

As discussed in the previous section, each element of the TME plays an important role in tumour progression and therapeutic resistance and, as such, its recapitulation within preclinical cancer drug discovery models is unequivocally important. However, many preclinical models fail to mimic the 3D and heterogenic complexity of the TME in the laboratory, resulting in inadequate drug screens and the selection of unsuitable agents for preclinical evaluations or clinical trials. This is a major contributing factor to the large number of costly pharmaceutical failures and long development timeframes. However, efforts to improve preclinical cancer models over the last three decades has seen an evolution of novel modelling techniques that carry unique advantages in drug discovery (Figure 6). The next section explores the current landscape and limitations of preclinical models for gynaecological cancer research.

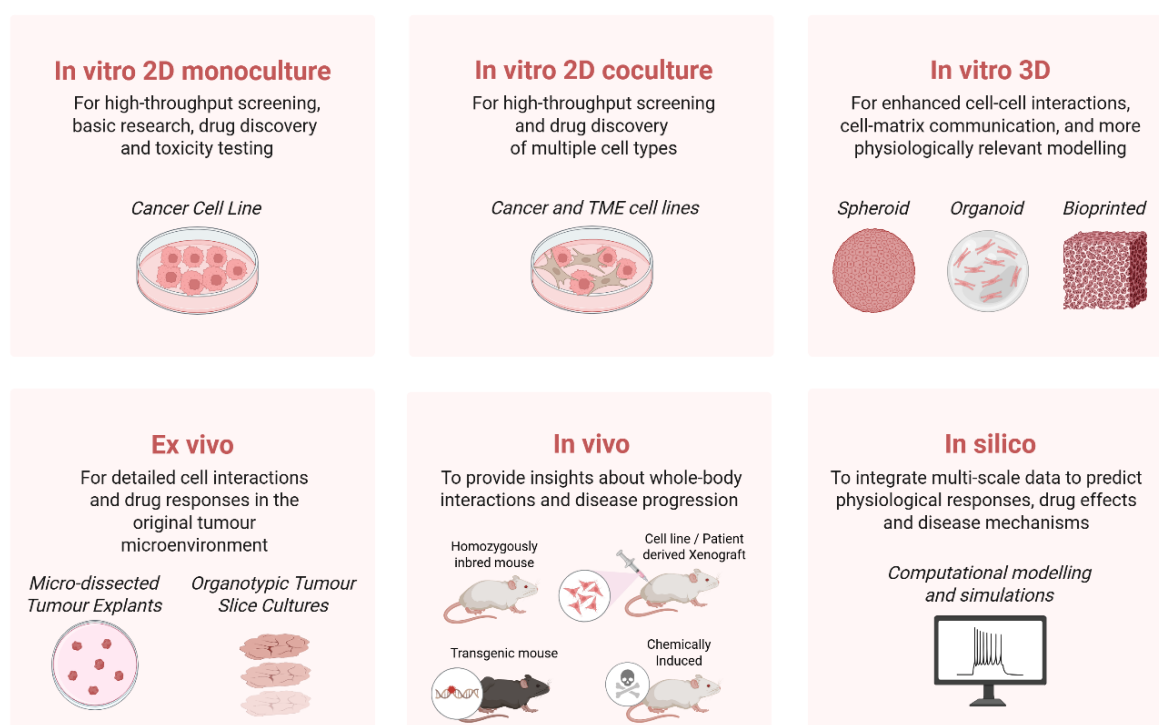


Figure 6. Preclinical models of cancer. Models range from 2D monocultures or co-cultures grown in cell culture plates as monolayers, in vitro 3D models (spheroids, organoids, bioprinted models), ex vivo explants and organotypic tumour slice cultures, in vivo mouse models and in silico models. Made in Biorender.

1.3.1 *In vitro*: Cell Culture

Since the 1990s, *in vitro* cancer cell lines have been a popular choice of drug screening model as they are affordable, high throughput, easily accessible and relatively simple to set up (Mirabelli et al., 2019). Consequently, a large number of pharmaceutical agents and concentrations can be screened at one time. Moreover, by utilising common commercial gynaecological cancer cell lines such as OVCAR8 and OVCAR3 (HGSOC) or ISHIKAWA and AN3CA (EC), research groups worldwide can replicate and build upon previous findings (Korch et al. 2012; Burkhard et al. 2025). This continuity is important for accelerating our understanding of cancer biology and the creation of novel therapeutic strategies (Figure 6).

However, one of the drawbacks to using commercial cell lines is that they are genomically unstable, exhibiting divergent events during long-term culturing – a phenomenon known as genetic drift (Quevedo et al. 2020). This results in the formation of subclone populations that are genomically and characteristically different to the original cell line e.g., changes to their morphology, proliferative behaviour and drug responses (Maru and Hippo, 2019; Quevedo et al. 2020). Moreover, it was found that many cancer cell lines do not genomically represent their corresponding primary tumour types. This issue is particularly acute in epithelial ovarian cancer, where numerous cell lines are poorly defined by disease origin and often exhibit molecular profiles that are inconsistent with their assigned labels (McCabe et al. 2023). This undermines their utility as subtype-specific disease models and limits the translational relevance of preclinical findings. While an effort has been made to utilise primary cultures of patient cancer cells, this method can be time-consuming, technically challenging and prone to culture establishment failures (Miserocchi et al. 2017). Importantly, simplistic monolayer cultures fail to reproduce the structural and cellular complexity of a native tumour and its TME, often being devoid of immune/stromal compartments and a 3D structure, resulting in inaccurate drug sensitivity data (Miserocchi et al. 2017).

1.3.2 3D models: Spheroids, Organoids, Tumour-on-chip, and Bioprinted Models

To overcome the limitations of 2D culture, a range of advanced 3D models has been developed, differing largely in their ability to mimic the physiological and structural features of the tumour and its TME, including spheroids, organoids, bioprinted models and tumour-on-chip (Figure 6).

(a) Spheroids

Spheroids are relatively simple 3D aggregates of cancer cells that form spontaneously when grown in media in low-attachment culture plates. They have been likened to the morphology of multicellular spheroidal clusters formed within the abnormal abdominal fluid of ovarian cancer patients, termed ascites (Al Habyan et al. 2018). While they are a cost-effective, high-throughput platform for studying the effect of drugs within a 3D cellular cluster, it is difficult to generate reproducible structures and sizes (Gunay et al. 2020). Moreover, while they play a role in understanding ascite-related biology, they are structurally and mechanistically very different to solid gynaecological carcinomas, being completely devoid of the TME (Al Habyan et al. 2018; Capellero et al. 2022).

(b) Organoids

Organoids are self-organising spheroid-like models formed by induced pluripotent stem cells or multipotent tumour cells upon the addition of growth factors, ECM components and/or scaffolds (Clark et al. 2022). They therefore contain heterogenous and genetically diverse tumour cell types, including some populations of the TME. They can also be derived from patient-derived tumours, maintained in culture for a relatively long time (1-2 months) and retain culture expandability, allowing for organoid lines to be bio-banked and reestablished for long-term assessment (Clark et al. 2022; Zhang et al. 2025). One study generated 56 ovarian cancer organoid lines from 32 patients that represented all histological subtypes of ovarian cancer. They showed interpatient heterogenic chemoresponses with some lines displaying chemoresistance and thus can serve as a predictive model prior to treatment selection (Kopper et al. 2019). However, organoids

rarely contain the full range of immune, vascular or ECM elements, and therefore limited in their ability to predict fibrotic, angiogenic or immunogenic responses (Zhang et al. 2025). Moreover, culture establishment can be slow and challenging, with some patient-derived cells being unable to be established in culture (Kopper et al. 2019).

(c) Bioprinted Tumour Models

Bioprinted tumour models are a relatively new concept that utilises computer-aided design printers to precisely place cancer, stromal, and extracellular matrix components (termed ‘bioinks’) in defined geometries (Tan et al. 2021; Mazzaglia et al. 2023). They offer advantages over organoids, such as quicker generation time, increased reproducibility, and increased establishment rates (Baka et al. 2023; Li et al. 2025c). Currently, bioprinted models for gynaecological research is a rapidly developing field, with numerous groups investigating the addition of stroma, vasculature and innervation into female reproductive bioprinted tissues (Yi et al. 2025). However, in bioprinted gynaecological *cancer* models, research has mostly centred around ECM incorporation. One study showed quick and reproducible generation of ovarian tumour-CAF coculture bioprinted models capable of *de novo* synthesis of ECM components, outperforming bioprinted tumour monocultures in terms of viability (Baka et al. 2023). Moreover, Shan *et al.* (2024) showed that increasing the stiffness of the ECM of bioprinted ovarian cancer models reduced their sensitivity to chemotherapy (Shan et al. 2024). However, there is an absence of studies which have bioprinted a gynaecological cancer model with a fully representative TME or demonstrated their efficacy in drug discovery or predictive screening (Yi et al. 2025).

(d) Tumour-on-Chip

Tumour-on-chip is a novel strategy in which cell lines, organoids, bioprinted structures or *ex vivo* tissue slices are cultured within a microfluidic chip system (Lim et al. 2025). This microfluidic chip incorporates microchannels that permit the replenishment of media, mimicking perfusion, shear stress, and biochemical gradients, and thus valuable for

studying drug penetration and delivery (Lim et al. 2025). These factors are particularly important in ovarian cancer, in which the fluidic stress of ascites can promote ovarian cancer metastases in the abdominal and peritoneal cavities. Moreover, it was shown that a dynamic environment could better predict *in vivo* chemo-responses than a static model (Marrella et al. 2021). However, tumour-on-chip are low throughput and technically demanding, often requiring both knowledge of advanced culture techniques (organoid, bioprinting, OTSC) and the development or accessibility to complex microfluidic equipment.

1.3.3 *In vivo* Animal Models

In vivo animal models in oncology encompass several major categories including genetically engineered (GEMM), syngeneic, chemically-induced and xenografted models, the latter comprising cell-line derived (CDX) and patient-derived (PDX) xenografts (Figure 6). They are widely used *in vivo* systems that are crucial for studying drug efficacy and toxicity in a more complex living organism prior to clinical translation (Day et al. 2015).

(a) GEMM, Syngeneic, and Chemically-induced Models

GEMM strains are generated by targeted genetic manipulation, inducing gene knockouts or mutations in genes that typically drive tumorigenesis like Trp53 mutations, Brca1/2 loss, and Pten deletion in HGSOc models, or Pten loss and Pik3ca gain-of-function mutations in endometrial cancer models (Joshi and Ellenson 2017; Zhai et al. 2017). While GEMMs offer a clinically relevant model for the study of early tumorigenic drivers and tumour biology, as well as drug efficacy in models harnessing similar genetic mutations, they are logistically complex and time-consuming due to requiring extensive breeding and with a long latency in tumour development of over a year in some cases (Table 8) (Zakarya et al. 2020). Complementing GEMMs are syngeneic models, which involve transplanting mouse-derived tumour cells into immunocompetent hosts of the same genetic background (Ahmed et al. 2024). In contrast, chemically-induced models utilise carcinogens to trigger *de novo* tumour formation, primarily serving the study of environmental carcinogenesis and preventative strategies (Kemp, 2015). All three mouse

models share one advantage which is that the host immune system remains intact, therefore they are uniquely suited for studying immunotherapy and T-cell dynamics (Table 8) (Day et al. 2015).

(b) Xenografted Models (CDX and PDX)

Compared with GEMM, gynaecological xenografted models offer a more efficient and technically accessible platform for oncology research. These models are established via heterotopic (subcutaneous) by orthotopic (intrauterine/ovarian) implantation of either immortalised commercial cell lines (cell line-derived xenografts; CDX) or patient-derived cells (patient-derived xenografts; PDX), with a typical latency period of 4-8 weeks (Table 8) (Liu et al. 2017; Yildiz et al. 2024).

While CDX models offer high reproducibility, PDX models maintain the genomic and phenotypic landscape of the original malignancy, providing a superior representation of intratumoral heterogeneity. Moreover, PDX enable the formation of tumours unique to individual patients, facilitating the development of personalised treatment strategies based on specific tumour responses (Abdollahi et al. 2022). Despite these advantages, PDX models are characterised by higher rates of engraftment failure compared to CDX.

The translational relevance of xenograft models is also heavily dictated by the site of engraftment. For instance, heterotopic subcutaneously injected models form tumours in an entirely different biological location, and consequently, the tumours receive different hormonal and biochemical cues found in the female reproductive tract (Yildiz et al. 2024). This discrepancy results in altered biological behaviour and ultimately poor predictive value during preclinical efficacy studies, with many promising pharmacological agents failing following their translation to clinical trials (Seyhan, 2019). In contrast, orthotopic xenografts better recapitulate the primary tumour niche, offering enhanced predictability of therapeutic response (Wen et al. 2024).

Xenografted tumours offer more advanced tumour architecture and cellular heterogeneity than other laboratory models such as 2D monolayers or 3D organoids by offering a highly organised environment of immune populations, blood vessels and 3D

structures (Figure 6) (Liu et al. 2017). However, to avoid host rejection increase the likelihood of successful engraftment, implantation is conducted in immunodeficient mice lacking mature T and B cells, ultimately misrepresenting immune-tumour biology (De Haven Brandon et al. 2020).

Moreover, in general animal models are very low throughput. Therefore, more throughput, high fidelity models which adequately recapitulate the patient's heterogenic tumoral-stromal landscape *ex vivo* are urgently needed. The ideal model should be rapidly generated and maintain the tumour's native architecture and cell-cell interactions, to fully ascertain drug sensitivity and resistance mechanisms, and consequently advance predictive drug studies.

Table 8. Comparison of *in vivo* animal models used for tumour biology and drug discovery studies

Model Type	Host Immune System	Tumour Origin	Applicability	Disadvantage
GEMM	Intact	Mouse (Genetic)	Tumour initiation and genetic drivers	Complex breeding; long latency periods; expensive
Syngeneic	Intact	Mouse	Immunotherapy and T-cell response	Limited to mouse biology; lacks the genomic complexity of human tumours
Chemical	Intact	Mouse (Induced)	Carcinogenesis and prevention studies.	Highly variable and unpredictable tumour onset, location and mutational profile
CDX (Cell-Line)	Immunodeficient	Human (Cell Lines)	High-throughput, initial drug screening	Minimal heterogeneity; poor clinical predictability; lack of immune context
PDX (Patient)	Immunodeficient	Human (Tumour Tissue)	Personalised medicine, heterogeneity	High engraftment failure rates; lacks functional adaptive immunity.

Section 1.4

3D *Ex vivo* Organotypic Tumour Slice Culture (OTSC) and Their Application in Gynaecological Cancer

Organotypic tumour slice culture (OTSC) has emerged as a 3D *ex vivo* platform that bridges the gap between oversimplified 2D cultures and resource-intensive *in vivo* models. The technique relies on the mechanical sectioning of live immobilised tissue into thin (200–300 µm) uniform slices using a high-precision vibratome, which can be rapidly established from patient or xenograft-derived material in just a few hours. On average, the slices can be cultured for up to 7 days and allow for in-depth exploration of drug responses using a range of downstream assays, including metabolic, cytotoxic transcriptomic and phenotypic evaluations (Figure 7) (Dewyse et al. 2021; Edwards et al. 2026).

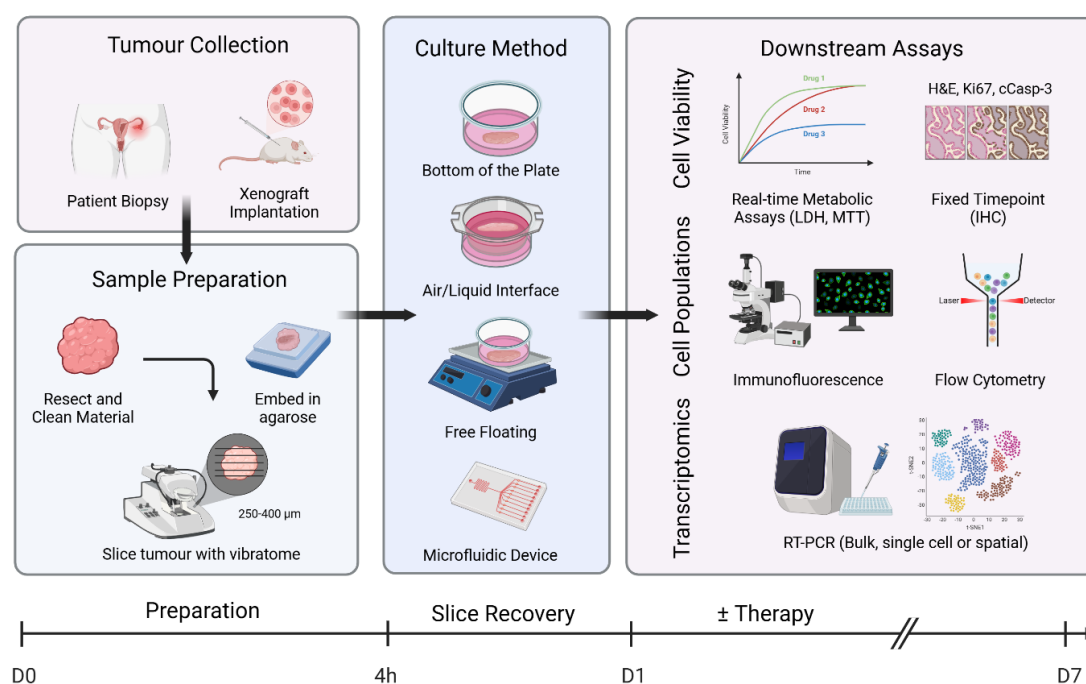


Figure 7. Schematic representation of the OTSC pipeline. 1) *Tissue Collection and Processing:* Live tumour tissue (patient biopsy or xenograft) is immobilised in agarose and sectioned using a high-precision vibratome to generate uniform slices. 2) *Ex vivo Cultivation:* Slices are maintained in specialised environments (e.g., air-liquid interface inserts or microfluidic devices) designed to preserve the native 3D architecture and oxygen gradients. 3) *Therapeutic Intervention:* Cultures are subjected to various treatments, including chemotherapy, targeted agents, or immunotherapies, and drug response is monitored via metabolic viability assays, proteomic profiling, and transcriptomics to evaluate cytotoxicity and TME-mediated resistance mechanisms. (From Edwards et al., 2026).

1.4.1 OTSC and the TME

The primary advantage of OTSC over 2D cultures, organoids or bioprinted models is the preservation of the native cellular heterogeneity, cell-cell contacts and histology of the original tumour and TME(Weitz et al. 2021). Studies have demonstrated OTSCs maintain the native stroma, including ECM, vasculature and immunological landscape (Sivakumar et al. 2019; Durante et al. 2025). For instance, a study by Sivakumar *et al.*, (2019) demonstrated the preservation of CD3, CD4, CD8, NK and B-cell lymphocyte lineages in patient-derived OTSC, similar to the original tumour bulk (Sivakumar et al. 2019). In addition, the slices maintain an oxygen gradient and a more complex 3D architecture, providing a more physiological relevant environment for drug absorption and drug delivery studies compared to 2D monocultures (Dewyse et al. 2021; Huang et al. 2023).

The inclusion of TME elements show significant disparities in drug efficacies between models during preclinical screens. For example, treatment with the anti-angiogenic agent vandetanib significantly reduced the viability of OTSCs but not tumour organoids – a difference attributed to variations in endothelial cell incorporation between the two models (Sivakumar et al. 2019). Interestingly, this system can also be used to explore the relationship between the ECM and immune cell activity in gynaecological cancer. For instance, one study used time-lapse confocal imaging in ovarian cancer slices and found T cell abundance and motility were lower in stiff, high collagen-dense regions, compared with more porous areas. Moreover, T cells rarely colocalised with tumour islets. Thus, OTSC offers further insights into how the TME can impact immune cell function and spatial distribution (Bougherara et al. 2015).

Slicing of patient-derived tumour resections also holds huge advantages, including maintaining patient tumour and TME heterogeneity and the underlying drug resistance mechanisms. Consequently, OTSC offers a platform for predictive drug response evaluations of individual patients that could be utilised for personalised medicine in the clinic. Moreover, patient biopsy overcomes the limitations of *in vivo* PDX models including host rejection, slow growth rates, and lymphocyte deficiencies, whilst minimising the use of animals (Huang et al. 2023; Edwards et al. 2026).

1.4.2 OTSC Technical Considerations

The success of OTSC in preclinical evaluations depends heavily on optimised protocols for slice preparation and culture maintenance (Edwards et al. 2026). Firstly, slice thickness must be carefully considered, typically falling within the range of 100-400µm. Thinner slices permit better oxygen diffusion, increasing their viability and longevity in culture, and facilitates downstream confocal microscopy imaging – which has an optimal laser resonance in tissue sections <250µm. In contrast, thicker sections are easier to slice and offers a larger snapshot of the tumour environment (Weitz et al. 2022).

To facilitate slicing, tumour explants must be cleaned of blood, fats and mucinous material which can stick to the vibratome blade and prevent adequate slicing. Prior to slicing, tumour resections can be biopsy punched into cores of set diameters (3-8mm) (Weitz et al. 2022; Huang et al. 2023). This ensures slice uniformity, increasing the reproducibility of downstream analyses. To improve immobilisation during slicing, the tumour biopsy can be embedded in agarose (typically 2-6%) and fixed to the slicing disc with cyanoacrylate glue. The agarose concentration is important, as it provides the necessary mechanical stability to match the tissue's intrinsic density. Moreover, slicing parameters must also be optimised for the tumour density. Typically, softer tumours require higher percentages of agarose, higher blade frequencies and amplitudes, alongside slower cutting speeds, compared with denser tumours.

Following sectioning, slices are maintained in diverse culture environments, including culture plates (static or rotating), inserts, or microfluidic “tumour-on-a-chip” devices to offer a more realistic physiological environment (Figure 7). Moreover, media can be optimised to the tumour subtype by adding growth factors, such as EGF and FGF, similar to their physiological environment (Weitz et al. 2022; Kayser et al. 2023; Edwards et al. 2026).

OTSCs are suited to are a wide range of downstream assays designed to capture a holistic view of drug responses. While metabolic assays (e.g., Resazurin or ATP bioluminescence) offer a high-level view of cytotoxicity, proteomic tools like flow cytometry, Western blot and IHC provide detailed data on cellular and molecular composition based on protein biomarkers. In addition, transcriptomic profiling (including single-cell, bulk and spatial

analysis) enables researchers to map the molecular landscape of the tissue, providing the mechanistic insights necessary to understand drug sensitivity and resistance mechanisms, facilitating drug discovery and predictive drug response profiling (Figure 7) (Weitz et al. 2021; Huang et al. 2023).

Despite the strengths of OTSC, this model remains a medium-throughput system limited to a yield of 15-30 slices per 1cm³ of tissue. Slices are also non-expandable in culture with a viability limited to 7 days, giving operators a very short timeframe to assess drug response. It is also technically challenging, requiring specialised equipment, tumour-specific optimised protocols, and access to xenograft or patient biopsy (Huang et al. 2023; Kayser et al. 2023).

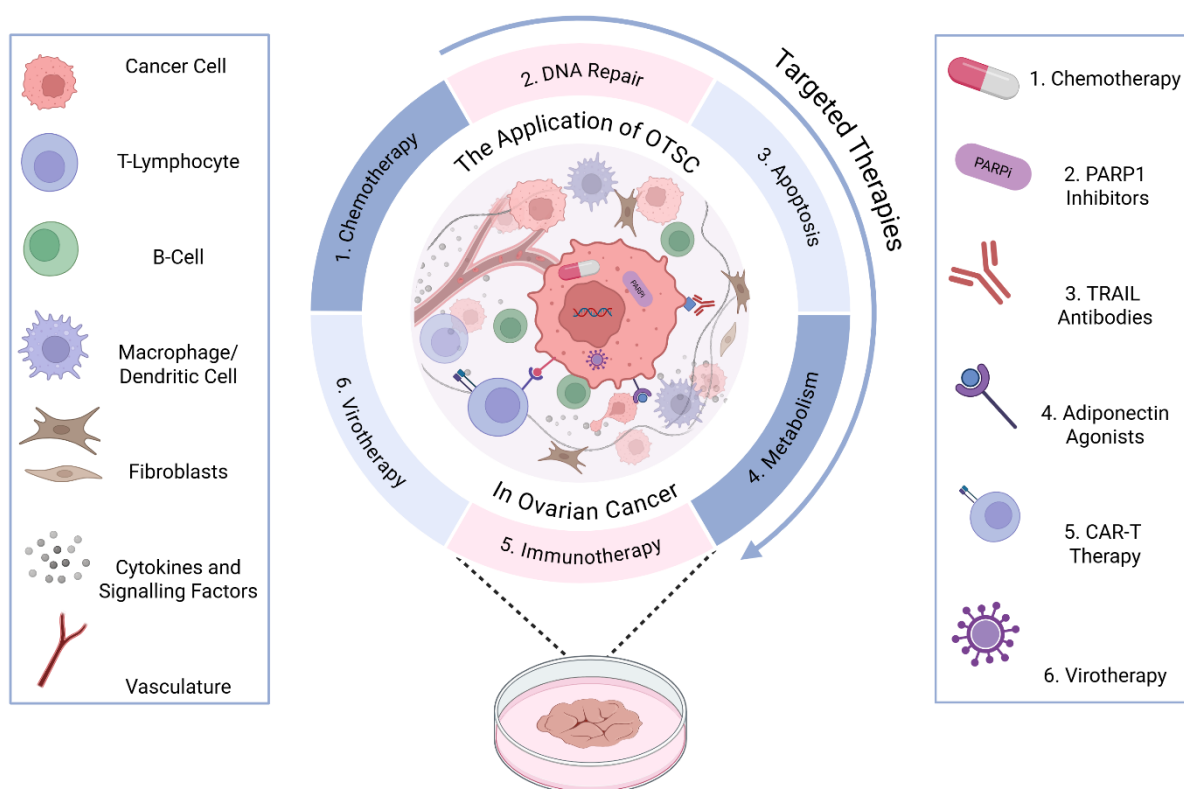


Figure 8. The application of Organotypic Tumour Slice Culture (OTSC) in ovarian cancer. OTSC has been used to explore the effects of chemotherapy, targeted therapeutics (DNA repair, apoptosis and metabolism), immunotherapy and virotherapy in xenograft and patient derived ex vivo slices which retain native cell-cell interactions and drug resistance mechanisms of the tumour microenvironment. From Edwards et al. (2026).

1.4.3 OTSC in Gynaecological Cancer Drug Evaluations

While OTSC has been explored frequently for numerous cancers including, breast, colon, pancreatic and melanoma, slices derived from ovarian cancer patient or xenografted models have only been utilised in ~16 studies over the last two decades (Table 9) and are virtually absent in endometrial cancer research. However, the available studies have tested chemotherapy, targeted therapeutics, and immunotherapies offering insights into OTSC's applicability in gynaecological cancer drug discovery and personalised medicine (Figure 8) (Edwards *et al.*, 2026).

(a) Chemotherapy

OTSCs have been used to evaluate responses to standard chemotherapies, such as carboplatin and paclitaxel. Studies consistently report substantial interpatient heterogeneity in drug sensitivity, with some patients showing minimal to no treatment response (Frederick *et al.* 2009; Brückner *et al.* 2021). Beyond interpatient variability studies, OTSCs have also been used to understand TME-mediated chemotherapeutic resistance mechanisms in ovarian cancer. When cisplatin was administered to ovarian cancer OTSC, immune fractions, including CD8+ and CD4+ lymphocyte levels, were unaffected. Instead, chemotherapy exposure induced varied PD-L1 expression in both immune cells and the stroma, depending on the patient (Dong *et al.* 2023). Similarly, Laforêts *et al* (2023) observed that while chemotherapy did not affect immune cell populations, it significantly altered T cell motility (Laforêts *et al.* 2023). These findings suggest that chemoresistance in ovarian cancer arises not only from intrinsic cellular mechanisms but also from immune suppression and physical stromal barriers, highlighting the necessity of *ex vivo* patient-derived models that maintain the original immune and ECM components.

(b) Targeted Therapy

Targeted therapies aim to exploit specific molecular vulnerabilities in tumours; however, their efficacy is strongly modulated by tumour heterogeneity and microenvironmental interactions. OTSC has been utilised to investigate DNA repair and apoptosis targeted strategies in ovarian cancer, demonstrating unique insights into the efficacy of novel

therapeutics that may overcome drug resistance mechanisms or challenge the standard of care (Figure 8). For example, resistance to PARP inhibitors (PARPi) can arise from an upregulation of transcriptional regulators, like FOXM1, or the Wnt signalling pathway. Novel combination approaches that target these resistance mechanisms showed higher efficacies than PARPi-monotherapy in the ovarian cancer OTSC platforms, offering novel proof-of-concept counteractive strategies in a clinically relevant model (Yamamoto et al. 2019; Brückner et al. 2021). Additionally, two apoptosis targeted therapies – the BH3-mimetic (ABT-737) that inhibits the activity of the antiapoptotic protein Bcl-xL and the DR-5 selective TRAIL variant (D269H/E195R) – showed greater cytotoxicity than the standard-of-care (Meijer et al. 2013; Lheureux et al. 2015). Thus, OTSC can offer insights into novel therapies that may outperform the standard of care.

(c) Immunotherapy

Due to OTSC's retention of a complex immunological landscape and signalling interactome, this slice platform is a relevant model for exploring immunotherapies, for example CAR-T therapy. When ovarian cancer OTSC slices were treated with FOLR1 CAR-T therapy, increases of CD8⁺ T cells and cytotoxicity in the tumour areas of OTSC slices were observed alongside elevations of pro-inflammatory cytokines Granzyme B, IFN- γ , and IL-2, compared with both untreated slices and slices treated with non-engineered T cells (Durante et al. 2025). Thus, OTSC can offer further insights into the underlying mechanisms of functional immunotherapeutic strategies. OTSC can also demonstrate how patient variability can alter CAR-T responses. For instance, cytotoxicity of OTSCs treated with HER2-mediated CAR-T therapy correlated with patient-specific HER2 expression levels. Moreover, the authors discovered that neo-adjuvant chemotherapy treated patients displayed less antigen expression and lower CAR-T response in OTSC slice experiments, compared to treatment naïve samples (Deshet-Unger et al. 2022). This highlights how the OTSC system can capture patient-derived variations at intrinsic (molecular) and extrinsic (therapeutic) levels and how this alters immunotherapeutic drug responses. Thus, OTSC could be an important *ex vivo* tool in biomarker-directed precision medicine pipelines.

Table 9. Therapeutics, slice and culture parameters, and downstream assays utilised in ovarian OTSC. From Edwards et al. (2026).

Year	Author	Therapeutic Strategy	Treatment	Specimens	Diameter (mm)	Width (µm)	Tissue Slicer	Culture method	Plate, media (per well)	OTSC Assays
2004	Kirby et al.,	Virotherapy	COX2 CRAAd	Hey xenografts, human primary OC	8.0	250	Krumdieck	Free-floating (60 RPM)	6-well plates, 2mL	RT-qPCR
2007	Rocconi et al.,	Virotherapy	CXCR4 CRAAd	Human primary EOC	8.0	250	Krumdieck	Free-floating	12-well plates	RT-qPCR
2007	Estes et al.,	Apoptosis	Anti-DR5 Antibody (TRA-8)	19 Human primary/secondary OC	5.0	300	Krumdieck	Bottom of the plate	24-well plate, 1.5 mL	Western blotting, ATP assay
2007	Tsuruta et al.,	Virotherapy	CRAAd	Human primary OC	8.0	250	Krumdieck	n/a	n/a	Luciferase assay
2009	Frederick et al.,	Apoptosis, Chemotherapy	TRA-8, carboplatin /paclitaxel	26 Human secondary OC	5.0	300	Krumdieck	Bottom of the plate	24-well plate, 1.5 mL	ATP assay, IHC
2013	Meijer et al.,	Apoptosis, Chemotherapy	rhTRAIL, DR5-TRAIL, Nutlin-3, cisplatin	5 Human primary OC	5.0	200-300	Krumdieck	Free-floating	6-well plate	H&E, IHC
2015	Lheureux et al.,	Apoptosis, Chemotherapy	ABT-73, carboplatin	25 HGSOC metastases	n/a	400	Vibratome	Bottom of the plate	n/a	H&E, IHC
2015	Bougherara et al.,	None	none	15 Human primary EOC	n/a	350	Vibratome	Air-liquid	35mm dishes, 1mL	Time-lapse IF
2019	Ramzan et al.,	Metabolic Targeting	AdipoRon	Human primary OC	n/a	300	Krumdieck	Bottom of the plate	n/a	H&E, IHC,
2019	Yamamoto et al.,	DNA Repair, Wnt Inhibition	Olaparib and Pyvinium Pamoate	1 Human primary HGSOC	n/a	n/a	Krumdieck	Bottom of the plate	n/a	Luciferase assay, H&E, IHC
2020	Riad et al.,	Theranostic Radiation	radioiodinated PARPi	Human HGSOC	n/a	300	Vibratome	Bottom of the plate	n/a	H&E, IHC,
2021	Dorrigiv et al.,	none	none	TOV112D and TOV21G xenografts	3.0	350	Mcllwain Chopper	Microfluidic device/ bottom of the plate	96-well plate	H&E, IF
2021	Brückner et al.,	DNA Repair, Chemotherapy	Carboplatin, Olaparib, Thiostrepton	14 Human primary OC	3.0	350	Mcllwain Chopper	Air-liquid	6-well plate	qPCR, H&E, IF
2022	Deshet-Unger et al.,	CAR-T Immunotherapy	ErbB2CAR T cells	29 Human primary HGSOC	n/a	250	Vibratome	Free-floating (70 RPM)	24-well plates, 0.5 mL	IFNγ ELISA, cytometry, H&E, IHC
2023	Dong et al.,	Chemotherapy	Cisplatin	Human primary OC	N/A	250-280	Vibratome	Air-liquid	n/a	H&E, IHC, qPCR
2023	Laforêts et al.,	Chemotherapy	Carboplatin/ Paclitaxel <i>in vivo</i>	HGS2/60577 xenografts, 17 primary HGSOC	n/a	350	Vibratome	Air-liquid	6-well plate	Time-lapse IF, IHC, cytometry

Abbreviations: ovarian carcinoma (OC), conditionally replicative adenovirus (CRAAd), death receptor 5 (DR5), Quantitative reverse transcription polymerase chain reaction (RT-qPCR), haematoxylin & eosin (H&E), immunohistochemistry (IHC), immunofluorescence (IF).

Section 1.5

Dopaminergic Signalling Pathways: Cancer Progression and Immunomodulation

Dopamine (DA) is the most predominant catecholamine neurotransmitter in the brain (Vallone et al. 2000). While DA is thought to predominately mediate processes including locomotion, cognition, mood, memory and hormone regulation in the brain, its functions in the peripheral nervous system, such as cardiac and renal vasodilation, are continually being elucidated (Urs et al. 2016; Han et al. 2019). Dysregulation of the dopaminergic system has been implicated in neurological disorders such as, Parkinson's disease, schizophrenia and bipolar disorder. However, recent evidence also implicates dopamine signalling in tumour biology (Urs et al. 2016; Tung et al. 2022). The next section will explore the dopaminergic signalling cascades, their role in gynaecological cancer progression, and the effect of dopaminergic modulators on tumour viability, angiogenesis and immunomodulation.

1.5.1 Dopamine Receptor Signalling

DA signals through five G protein-coupled dopamine receptors (GPCRs), categorised into two subfamilies: D1-like (DRD1 and DRD5) and D2-like receptors (DRD2, DRD3 and DRD4) (Undieh 2010). The receptors differ in their homology, ligand affinity and abundance. For example, D1-like receptors (DRD1 and DRD5) exhibit high levels of amino acid sequence conservation, particularly in their transmembrane domain where they display 79% homology between members (Grandy et al. 1991). Whereas functional divergence between DRD1 and DRD5 primarily arises from differences in tissue expression patterns and ligand affinity; DRD1 is more abundantly expressed than DRD5, whereas DRD5 binds to dopamine with 10-times greater affinity than DRD1 (Grandy et al. 1991; Rosas-Cruz et al. 2021). Similarly, D2-like receptors, DRD3 and DRD4 are also less abundantly expressed but bind dopamine 10-times greater than DRD2 and have 75% and 53% transmembrane receptor homology to DRD2, respectively (Rondou et al. 2010; Rosas-Cruz et al. 2021). As a result, pharmacological agents targeting DRD1 frequently exhibit cross-reactivity with DRD5, and those targeting DRD2 can also engage with DRD3 and DRD4. Signalling through the different subfamilies alters downstream mechanisms, including adenylyl cyclase activation or inhibition (Vallone et al. 2000). These signalling events regulate neuronal excitability, as well as key cellular processes including cell proliferation, survival, migration, and motility.

1.5.2 D1-like Signalling

D1-like receptors are bound to a heterotrimeric G protein primarily consisting of stimulatory G alpha (G_{α_s}), G_{β} and G_{γ} subunits (Figure 9). Upon dopamine engagement, the receptor undergoes a conformational change enabling it to act as a guanine nucleotide exchange factor (GEF), exchanging GDP for GTP in the G_{α} subunit (Teng et al. 2022). This results in its activation and dissociation from the $G_{\beta\gamma}$ complex. Activated G_{α_s} directly stimulates adenylyl cyclase, leading to an increase in intracellular cyclic adenosine monophosphate (cAMP) levels. Elevated cAMP activates protein kinase A (PKA), which subsequently phosphorylates a wide range of downstream substrates, including ion channels, transcription factors, and cytoskeletal proteins (Jones-Tabah et al. 2022). One key target of PKA is the cAMP response element-binding protein (CREB), whose phosphorylation facilitates transcriptional activation of genes involved in cell survival, metabolism, and differentiation (Figure 9) (Rivera-Maya et al. 2024). Moreover, the liberated $G_{\beta\gamma}$ dimer can also bind and activate PI3K to increase the production of PIP3, that in turn activates Akt and its downstream effector mTORC1, driving proliferation and increased metabolism (Nürnberg and Beer-Hammer 2019; Wijayaratna et al. 2023).

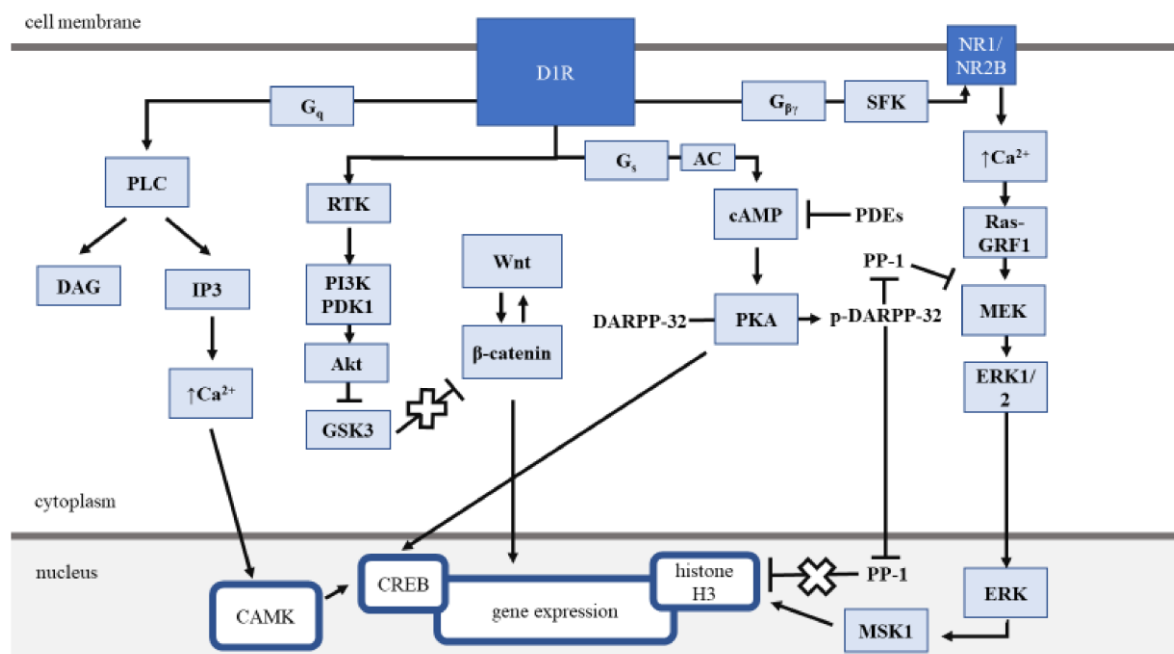


Figure 9. DRD1 signalling in cancer. DRD1 couples with various G-proteins (G_s , G_q , $G_{\beta\gamma}$) to initiate downstream cascades including cAMP/PKA, PI3K/AKT, Wnt/ β -catenin, MAPK/ERK thereby sustaining nuclear signalling and gene expression. From Sobczuk et al. (2020).

1.5.3 D2-Like signalling:

In its inactive state, D2-like receptors are bound to the inhibitory G protein complex ($G_{i/o}$) which differs to D1-like receptors by containing an inhibitory G alpha subunit ($G_{\alpha i}$) (Tung et al. 2022). Dopamine-receptor engagement leads to GEF activity, causing the dissociation of $G_{\alpha i}$ -GTP from the $G\beta\gamma$ dimer and the subsequent binding and inhibition of adenylyl cyclase (Figure 10). This prevents the conversion of ATP to cAMP and the activation of protein kinase A. This subsequently inhibits the phosphorylation of PKA's downstream effectors, such as Raf-1, leading to its activation and increased MEK/ERK signalling. Similarly, the dissociated $G_{\alpha i}$ and $G\beta\gamma$ dimer can also independently stimulate Raf/MEK/ERK kinase effectors, causing the activation of cell proliferation and survival pathways (Dhillon et al. 2002; Chocarro-Calvo et al. 2012; Zhang et al. 2022b).

1.5.4 β -arrestin-dependent signalling (alternative pathway)

Interestingly, D2-like receptors also form a strong interaction with the scaffold protein β -arrestin-2 triggering an alternative pathway independent of G protein activity, termed β -arrestin-dependent signalling, that has been implicated in cancer (Urs et al. 2016). β -arrestin-2 binds to the phosphorylated sites on the D2R intracellular loops causing these scaffold proteins to undergo a conformational change, revealing binding sites for kinases (Figure 10) (Han et al. 2019). For instance, β -arrestin-2 can scaffold the key effectors of the MAPK pathway including, Raf (MAPKKK), MEK1/2 (MAPKK) and ERK1/2 (MAPK), holding these kinases together and leading to increased activation and signal transduction (Figure 10) (Urs et al. 2016; Han et al. 2019).

Phosphorylated ERK (p-ERK) leads to the phosphorylation and ubiquitination of pro-apoptotic proteins BIM and BAD leading to increased cell survival, as well as p-ERK translocation into the nucleus and the activation of genes such as c-myc, involved in increased cell growth, metabolism and proliferation (Ma et al. 2021). β -arrestin 2 can also scaffold PI3K/AKT/mTOR (regulates autophagy), Toll-like receptors 4 (TLR4)/NF- κ B (inflammation) and Wnt signalling proteins (Han et al. 2019). Hence, uncontrolled signalling through the β -arrestin-dependent pathway can lead to aberrant tumorigenic processes.

β -arrestin also plays a key role in DRD2 receptor internalisation and receptor recycling by binding directly with clathrin and adaptin protein 2, forming a complex necessary for GPCR translocation (Tung et al. 2022). Hence, this alternative pathway can both enhance DRD2 downstream signal transduction and negatively regulate D2R signalling through receptor endocytosis (Tung et al. 2022). Excess D2R receptor trafficking and downregulation may also lead to increased dopamine-D1R receptor engagement and increased activation of MAPK signalling.

1.5.5 Dopaminergic Dysregulation in Gynaecological Cancer Progression

While dopamine and its receptors are expressed predominantly in the brain, they are also present in a wide range of non-neuronal cell types, including immune, endothelial and cancer cells (Moreno-Smith et al. 2013; Grant et al. 2022; Wu et al. 2025). Moreover, because dopaminergic signalling is known to drive pro-tumorigenic pathways that are often dysregulated in cancer, such as Ras/PI3K/AKT and Raf/MEK/ERK, dopaminergic involvement as an upstream driver of cancer has been further investigated (Weissenrieder et al. 2019).

Exploration of dopamine receptor expression and gynaecological cancer severity have indicated that dopaminergic dysregulation could be involved. Firstly, increased DRD2 expression is significantly associated with poorer endometrial cancer grades, stages and aggressive histology subtypes (e.g., serous), as well as associated with worse progression free survival and overall survival rates (Pierce et al. 2021; Español et al. 2023). Moreover, 38 dopamine-related mRNA transcriptomes were changed between endometrial cancer samples and controls; interestingly, decreased expression of DRD5 and COMT (the enzyme responsible for dopamine, norepinephrine, and epinephrine metabolism) with an increase in DRD2 levels were found in all endometrial cancer samples regardless of stage, compared to controls. These findings were supported by the same patterns in protein expression in patient sera (Czerwiński et al. 2021). Dopamine receptor involvement in ovarian cancer appears to be more heterogenous; a number of studies have found DRD2 expression in ovarian cancer cell lines, while another study reported only 50% of ovarian cancers had high DRD1 and/or high DRD2 expression (Yong

et al. 2017; Peters et al. 2019). Moreover, a high level of the DRD2 scaffold protein, β -arrestin-2, is a survival prognostic factor in ovarian cancer (Czogalla et al. 2020). However, the relationship between dopamine receptor expression and ovarian cancer stage, grade, subtype and survival rates remains unclear.

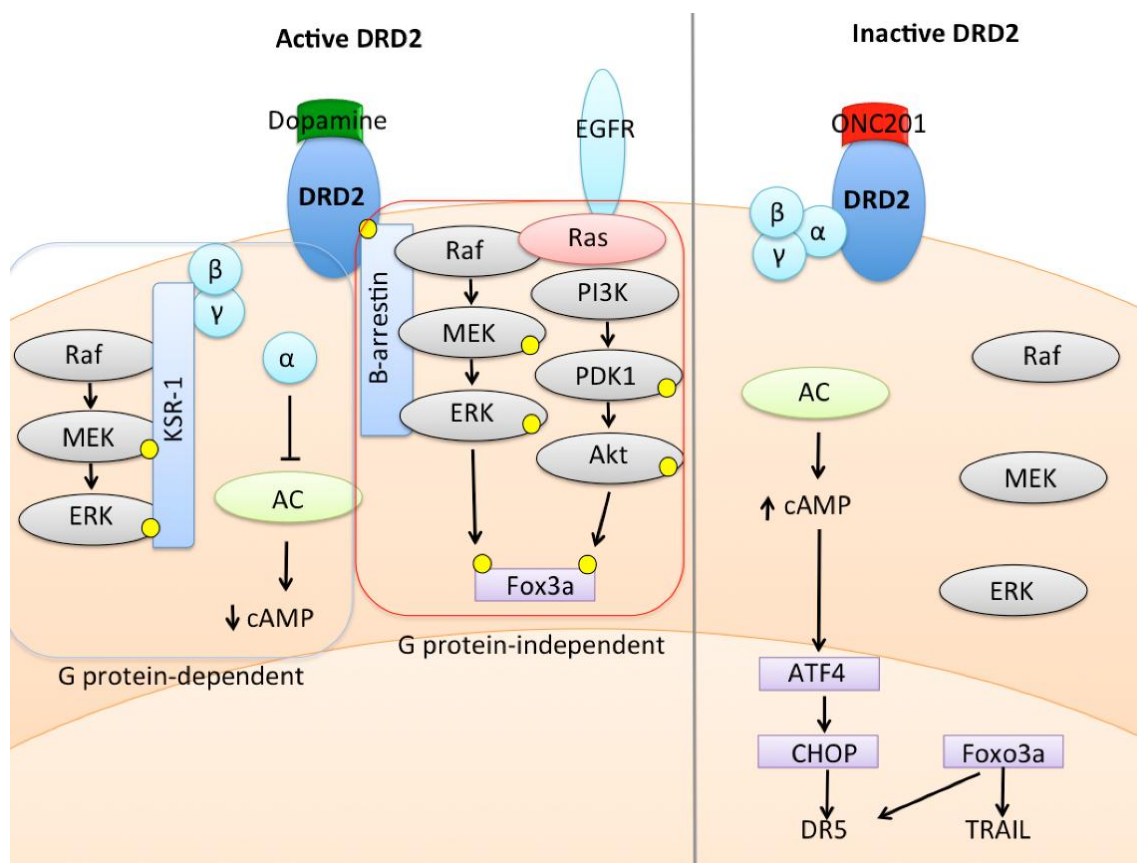


Figure 10. DRD2 signalling in cancer. Under active conditions (stimulated by dopamine), DRD2 utilises β -arrestin and G protein pathways to activate ERK and Akt, effectively silencing the tumour suppressor Foxo3a. Conversely, inhibition of DRD2 (e.g., via ONC201) halts these cascades. It has been suggested that ONC201 inhibition drives ATF4/CHOP/DR5 stress-induced apoptotic pathway and TRAIL-mediated cell death. From Oncocotics (<https://www.rdworltonline.com/the-role-of-drd2-in-cancer>).

1.5.6 DRD1 Agonism and DRD2 Antagonism in Gynaecological Cancer and the TME

Early evidence linking dopaminergic modulation to anti-cancer effects came from epidemiological studies; one meta-analysis investigating lung cancer risk in 348,780 Parkinson's disease (PD) patients found they had a 47% reduction in risk of developing the malignancy (Xie et al. 2016). Further interrogation into this finding identified that only patients diagnosed with PD and schizophrenia – and thus prescribed dopaminergic modulators such as the dopamine mimetic L-DOPA and antipsychotics – were associated with this decreased risk (Tung et al. 2022). These discoveries led to the hypothesis that dopaminergic modulators could harness anti-cancer benefits and thus, numerous investigations on the effects of anti-psychotics on cancer progression have been conducted.

Administration of dopamine and synthetic modulators have demonstrated anti-cancer properties, including TME-mediated changes such as anti-angiogenesis and anti-fibrosis (Basu et al. 2001; Haak et al. 2019; Alam et al. 2025). Basu et al. (2001) reported that dopamine, a DRD2 agonists (bromocriptine and quinpirole) downregulated angiogenesis in ovarian cancer *in vivo* models, reducing endothelial cell proliferation and migration (Basu et al. 2001). Similar findings were reported by Moreno-smith *et al.* (2013) who found DA-DR1 increased pericyte coverage on tumour vasculature and DA-DR2 signalling exhibited significantly reduced microvessel density, resulting in inhibited ovarian tumour growth *in vivo* (Moreno-Smith et al. 2013). DRD1 agonists also inhibited YAP/TAZ signalling in mesenchymal cells reversing fibrosis and reducing ECM stiffness – this could yield benefits in cancer stroma in which ECM stiffness has been linked to poor drug penetration and immune cell migration in the TME (Haak et al. 2019).

Similar results were found with DRD2 blockades; Thioridazine induced apoptosis and autophagy in ovarian cancer cell lines and inhibited the growth of xenografted mouse models (Yong et al. 2017). Moreover, the first-in-class imipridones, ONC201 and ONC206, are also selective antagonists of D2-like receptors that have also shown anti-cancer effects in numerous cancer types, including colon cancer, glioblastoma models, and recently gynaecological cancers (Ishida et al. 2018). ONC201 is known to inactivate AKT/ERK signalling and induce TRAIL and death receptor 5 gene expression (Wagner et al. 2017). When this drug was tested in ovarian cancer cell lines both AKT/mTOR/S6 and

MAPK pathways were inhibited, alongside a reduction in cell proliferation and induced apoptosis and G1 phase cell cycle arrest. Moreover, in transgenic models, ONC201 reduced tumour weight and reduced serum VEGF production, suggesting a role in anti-angiogenic pathways (Fan et al. 2022). When ONC201 and ONC206 were tested in uterine serous endometrial cancer cell lines, both drugs reduced viability in a concentration-dependent manner with concomitant disruption of p38MAPK/ERK signalling pathways (Figure 10) (Hu et al. 2020a). Moreover, ONC206 also significantly reduced tumour burden and proliferative ki-67 expression in xenografted endometrial cancer mouse models (Hu et al. 2020a).

1.5.7 Dopaminergic Immunomodulation

There has been an increasing amount of evidence reporting dopamine receptor targeting as a novel immunotherapeutic strategy, with studies indicating numerous immune subtypes express DRD1-5 on their cell surface and that DRD1 agonists and DRD2 antagonists have immune-activating effects in *in vitro* and *in vivo* cancer models (Zhang et al. 2017) (Moreno-Smith et al. 2013; Yong et al. 2017; Zhang et al. 2017).

(a) Lymphocytes

T effector lymphocytes are essential for anti-tumour immune responses. However, T cells, particularly CD8⁺ T cells, can become exhausted and non-functional in the TME, even in the presence of antigen. Signalling of dopamine through DRD5 in CD8⁺ T lymphocytes restored T cell cytotoxicity functionality and promoted their differentiation into tissue-resident memory T cells (CD103⁺) – a phenotype associated with longer survival and disease regression in colorectal cancer (Chen et al. 2022). These effects were completely blocked by administering D1-like receptor antagonist SCH23390 and by siRNA *DRD5* knockdown in CD8⁺ T cells, but not by DRD2 antagonism or *DRD1* silencing, suggesting dopamine-DRD5 stimulation is a critical mediator of this immune process (Chen et al. 2022). Similarly, DRD5 is also highly expressed in B cells and dopamine-DRD5 signalling reprogrammes them to become potent activators of CD8 T cells, associated with enhanced JAK/STAT signalling and an upregulation of genes involved in B cell motility, antigen presentation, and activation (Wu et al. 2025). Similarly, D1-like

receptor (DRD1/DRD5) activation with agonist SKF38393 also increases NK cytotoxicity, with effects associated with increased cAMP-PKA-CREB signalling, upregulation of IFN- γ and anti-tumour effects against lymphomas, whereas D2-like activation with agonist quinpirole suppresses NK function (Zhao et al. 2013). Together these studies indicate that DRD1 agonism and/or DRD2 antagonism could have anti-tumour effects via lymphocyte immunomodulation.

(b) Myeloid Cells

DRD1 agonism and DRD2 antagonism could also be beneficial in stimulating anti-tumour behaviours in myeloid cells. For example, recent studies show DRD3 antagonism in DCs boosts antigen cross-presentation to CD8⁺ T cells, a mechanism associated with anti-tumour activity (Figueroa et al. 2017). In lung and melanoma tumours, dopamine signalling via D1-like receptors (DRD1/DRD5) suppresses the inhibitory function of monocytic MDSCs *in vivo* resulting in T cell proliferation and activation, and these effects were blocked by D1-like antagonists and induced by agonists (Wu et al. 2015).

In contrast, in neutrophils, the DA-DRD5 axis appears to inhibit several neutrophil functions, including migration, activation-induced morphological changes, and reactive oxygen species production. Dopamine's inhibition of neutrophil migration is lost in DRD5 knockout mice, indicating that contradictory to other myeloid cells subtypes, DR1-like antagonism could be efficacious in immune activation.

Dopamine receptors are also highly expressed in macrophages and dopaminergic modulation in this cell type has shown interesting results. M2 macrophage polarisation is considered a pro-tumorigenic phenotype associated with immunosuppression, cancer progression and angiogenesis. Interestingly, dopamine signalling has also been associated with this process. When dopamine signals through DRD2, pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) were reduced in M1-polarized macrophages in pancreatitis, and DRD2 antagonists block this suppressive effect, enhancing M1 features. Moreover, in a myeloid-specific *DRD2* knockout mouse model, there was an increase in the pro-inflammatory M1 phenotype, again showing DRD2 blocking favours M1 phenotypes (Han et al. 2020). Similarly, D1-like receptor activation by the D1-agonist Fenoldopam and DA-DRD5 signalling promoted a proinflammatory macrophage response (Bone et al. 2017;

Nolan et al. 2018). Given that gynaecological cancers are associated with a pro-tumour M2 phenotype, both DR2 blockades and DR1 activators in macrophages could be beneficial for M1 polarisation and immune activation. However, contradictory to this, DA-DRD2 signalling (agonism) has been implicated in M2 to M1 polarisation in glioblastoma, and DA-DRD5 signalling in M1/M2 polarisation via CREB activation/NF- κ B inhibition in colitis (Qin et al. 2015; Liu et al. 2021a). Discrepancies could be due to utilising isolated immune cell populations *in vitro* rather than advanced TME co-culture models, therefore, more research needs to be conducted in advanced immune-tumour models to truly understand the impact of dopaminergic signalling in the TME.

Table 10. DR1-like and DR2-like receptor expression in immune cells. Adapted from Zhang et al. (2017).

<i>Immune cells</i>	<i>Dopamine Receptors</i>
<i>Effector T cells</i>	DRD1 ^{low} , DRD2, DRD3, DRD4, DRD5
<i>Regulatory T cells</i>	DRD1, DRD3, DRD5
<i>B cells</i>	DRD1, DRD2, DRD3, DRD4, DRD5
<i>NK cells</i>	DRD1, DRD2, DRD3, DRD4, DRD5
<i>Monocytes</i>	DRD2, DRD3
<i>Macrophages</i>	DRD1, DRD2, DRD3, DRD4, DRD5
<i>Dendritic cells</i>	DRD1, DRD2, DRD3, DRD4, DRD5
<i>Neutrophils</i>	DRD1, DRD2, DRD3, DRD4, DRD5

Section 1.6

Nanotherapeutic Strategies in Gynaecological Cancer

1.6.1 Nanoparticles

In the last decade, nanomedicines – small molecules (1-300nm) manipulated to have a variety of shapes, sizes, and physicochemical properties – have become increasingly popular in anti-cancer formulations, with many receiving clinical approval for the treatment of cancer (Tong et al. 2024; Zhang and Tian 2024). One attractive quality of nanoparticles is that they coat and encapsulate drugs, consequently improving their water-solubility, lipophilicity, renal clearance and pharmacokinetics (Peralta-Cuevas et al. 2026). Similarly, they can be engineered to both target cells and release their cargo under certain physiological conditions, for example through the utilisation of thermal sensitive linkers or pH-opening carriers (Amin et al. 2020; Tapponi et al. 2025). Moreover, their small size also facilitates tumour uptake through enhanced permeation from leaky tumour vasculature via the enhanced permeability and retention (EPR) effect (Gralewska et al. 2024). Numerous materials have been explored for drug encapsulation, including lipids, polymers, metals, and silica, each bringing unique advantages for drug delivery (Figure 11) (Gralewska et al. 2024). Another interesting aspect of nanomedicines is that the nanoparticle shell can be conjugated with cancer-targeting moieties to enhance the delivery of the encapsulated cargo (Zhao et al., 2022). This enables enhanced on-target drug delivery and reduces off-target effects.

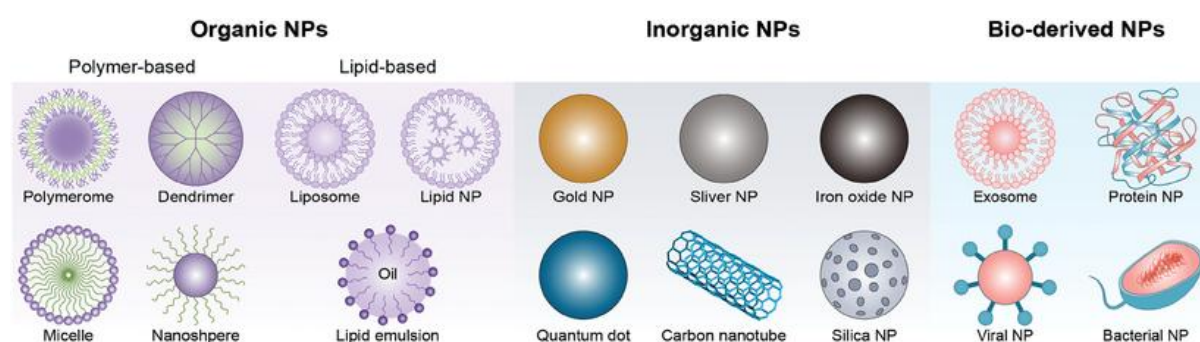


Figure 11. Types of nanomedicines. Nanoparticles are categorised based on their carrier material and include Organic (polymeric, lipids), Inorganic (metals, silica, carbon), and Bio-derived (protein, viral, bacterial). From Zheng et al. (2024).

Nano-formulation strategies hold huge potential for drug repurposing in oncology, in which many existing medications may exhibit anti-cancer potential but are limited in their efficacy due to poor solubility, rapid metabolic clearance, low tumour penetration or off-target toxicity. Hence, their nanoencapsulation could prove effective in overcoming biological and pharmacological barriers (Elmahboub et al. 2025). Moreover, nanomaterials offer a vector in which to co-deliver multiple drugs. As our knowledge expands of the mechanisms of oncologic drug resistance, either through intrinsic or TME-mediated signalling compensations, multidrug targeting is becoming an increasingly popular field. While simultaneous co-delivery of free drugs has spatiotemporal challenges, these could be rectified with their co-encapsulation within a drug delivery system (Ahmadi-Nouraldin et al. 2024; Shao et al. 2025). It is important to note, targeting multiple cell types or signalling pathways may inadvertently affect normal cellular homeostasis, increasing the risk of off-target effects (Li et al. 2024). This again highlights the need for advanced *ex vivo* drug discovery models which incorporate all the drug resistance mechanisms of the original tumour and TME and allow for in depth exploration of nano-biodistribution and toxicology studies in order to fully ascertain the efficacy and risks of novel nanomedicines.

1.6.2 Nanomedicines for Gynaecological Cancers

Lipid-based systems represent the most clinically advanced nanomedicine approach in gynaecology, with pegylated liposomal doxorubicin (Caelyx®) already approved for ovarian cancer treatment. Caelyx demonstrated enhanced efficacy over free doxorubicin, exhibiting prolonged circulation, slower drug release profiles, reduced cardiotoxicity and enhanced tumour penetration through the EPR effect (Gabizon et al. 2025). However, repeated dosing of PEGylated nanoparticles (like Caelyx) can trigger the production of anti-PEG IgM antibodies, leading to much faster clearance rates on the second or third dose (Fu et al. 2025).

Table 11. Clinical trials for nanomedicines for ovarian and endometrial cancers.

Trial Identifier	Agent	Cancer Type	Phase	Status	Nanoformulation
NCT05123482	AZD8205	Ovarian & Endometrial	I/II	Recruiting	ADC Nano-conjugate: B7-H4 targeted topoisomerase inhibitor.
NCT06915025	IMNN-001	Ovarian	III	Recruiting	DNA Plasmid Lipid Nanoparticle: IL-12 gene delivery
NCT05325229	Nab-Docetaxel	Ovarian	II	Active	Albumin-bound Nanoparticle of Docetaxel
NCT05257408	Nab-Paclitaxel	Ovarian	III	Active	Albumin-bound Nanoparticle of Paclitaxel
NCT07166094/ NCT06619236	Rina-S	Ovarian & Endometrial	III	Active	ADC Nanoconjugate: FR α -targeted topoisomerase inhibitor
NCT07224464	Sarah (SaNP)	Solid Tumors	I	Recruiting	Magnetic Nanoparticle: Iron-oxide core for hyperthermic destruction.
NCT03348631	Tazemetostat	Ovarian & Endometrial	II	Active	Nanoparticle delivery (EPZ): Targeted EZH2 inhibitor delivery.
NCT04163094	W_ova1	Ovarian	I	Terminated	Liposomal mRNA Vaccine: Encapsulated mRNA to induce immune response.
NCT05377996	XMT-1660	Ovarian & Endometrial	I/II	Recruiting	Dolasynten Scaffold: High-load B7-H4 targeted nano-scaffold.

The clinically approved albumin-bound paclitaxel nanoparticle Abraxane has also been used off-label for ovarian and endometrial cancers demonstrating improved adverse reactions in patients who were previously experiencing paclitaxel-induced hypersensitivity (Fader and Rose 2009). As seen in Table 11, several nanoformulations have entered clinical trials for gynaecological cancers, however, most remain in preclinical development (Zhang and Tian 2024). This is due to major barriers, such as complex manufacturing and scalability, heterogenous EPR effect across *in vivo* models and patients, accelerated blood clearance upon repeated dosing, limited *in vivo* stability or toxicity, and regulatory challenges (Tong et al. 2024).

1.6.3 Polymeric Nanoparticles

Polymeric nanoparticles, such as the FDA-approved poly(lactic-glycolic) acid (PLGA), are another frequently used material in nanomedicine due to their high biocompatibility, low immunogenicity, and predictable degradation into natural byproducts (carbon dioxide and water), reducing the risk of adverse reactions during nanomaterial metabolism (Figure 12). This material is also very effective in encapsulating hydrophobic drugs, such as paclitaxel.

Both different molecular weight chains of PLGA and ratios of lactic and glycolic acids can be modified to alter the polymer's pharmacokinetics and mechanical properties. For example, shorter PLGA chains are quicker to hydrolyse resulting in quicker degradation rates and rapid drug release. Similarly, higher ratios of glycolic acid, such as the 50:50 ratio, demonstrate one of the fastest drug release profiles (2-6 weeks). This is because glycolide content increases the hydrophilicity of the material, consequently enhancing its water uptake and hydrolysis (Jumadi et al. 2025). However, due to the carboxyl groups (COO^-) of the copolymer, PLGA typically has a negative charge which can reduce cellular uptake at the negatively charged cell membrane (Figure 12) (Danhier et al. 2012). Moreover, the outer surface of PLGA displays levels of hydrophobicity, resulting in nanoparticle aggregation and opsonisation – a process caused by the binding of plasma proteins to the PLGA surface in the form of a protein corona. This can lead to decreased drug uptake and increased immune clearance (El-Hammadi and Arias 2022).

To reduce surface charge, hydrophobicity, and opsonisation, the PLGA can be functionalised with Polyethylene Glycol (PEG). PEG creates a hydrophilic layer around the PLGA nanoparticles, increasing its surface charge ($\sim$ neutral) and reducing aggregation and protein absorption (Figure 12). This in turn increases circulation time, reduces proteolytic degradation and slows immune clearance (El-Hammadi and Arias 2022; Spinelli et al. 2025). However, the triggering of anti-PEG antibodies may reduce immune compatibility (Fu et al. 2025).

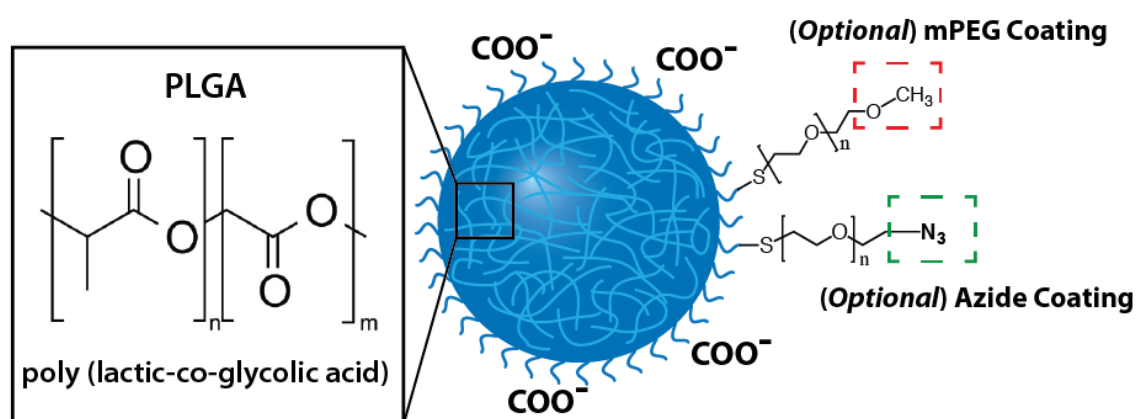


Figure 12. Structure of poly(lactic-co-glycolic acid) (PLGA) nanoparticles. PLGA nanoparticles are formed from polymeric chains of lactic acid and glycolic acid units. Ratios of these units can be altered to change degradation rates. PLGA nanoparticles can be functionalised with Polyethylene glycol (PEG) or azide to improve the surface charge and pharmacodynamics. From: Lunanano.

Another strategy to improve delivery of PLGA nanoparticles is through the conjugation of cancer-specific ligands. In ovarian cancer, numerous receptors are overexpressed at the cell surface, including HER2, CD44 and FR- α (Li et al. 2025b). These can be targeted by the functionalisation of receptor-specific antibodies (e.g., Herceptin for HER2), or the receptor's native ligand such as hyaluronic acid for CD44 or folic acid for FR- α (Wang et al., 2023). For example, CD44-targeting PLGA nanoparticles co-delivering paclitaxel and FAK siRNA have shown significant tumour growth inhibition in chemoresistant ovarian cancer models, including patient-derived xenografts (Byeon et al. 2018). However, to date, no PLGA nanoparticles have been approved for the treatment of ovarian or endometrial cancer.

Section 1.7

Aims and Hypotheses

In gynaecological cancers, the dopamine D2 receptor (DRD2) is frequently overexpressed, correlating with aggressive phenotypes and poor patient outcomes. Moreover, dopaminergic modulating agents such as D1-like agonists and D2-like antagonists demonstrate anti-cancer, anti-angiogenic and immune-stimulating effects in *in vitro* and *in vivo* models. However, exploring this pathway as a therapeutic target requires moving beyond models that fail to capture the cellular "crosstalk" of the native environment and/or patient-specific drug resistance mechanisms.

This PhD project seeks to bridge the translational gap in gynaecological oncology by integrating 3D tumour microenvironment (TME) modelling with targeted drug repurposing. By utilising Organotypic Tumour Slice Culture (OTSC), this research evaluates the potential of DRD2 blockade to not only induce cytotoxicity in malignant cells but also to activate the immune system in the immunosuppressed TME. Finally, to address the pharmacological hurdles of systemic delivery, this work explores nano-engineering solutions to optimise the therapeutic index of our lead candidate, Domperidone.

1.7.1 Aims

- 1) To develop, characterise and validate a high-precision OTSC platform for gynaecological cancer.
- 2) To profile the dopaminergic interactome and screen lead modulators in 2D models.
- 3) To characterise the mechanism of the lead candidate Domperidone
- 4) To validate the anti-tumour and immunomodulatory efficacy of Domperidone in a 3D environment.
- 5) To engineer a nanoparticle delivery system to enhance the therapeutic window of Domperidone.

1.7.2 Hypotheses

- 1) The OTSC model will preserve the native architecture and cellular heterogeneity of High-Grade Serous Ovarian Cancer (HGSOC) and Endometrial Cancer (EC), maintain tissue viability up to 7 days in culture, and demonstrate more accurate chemotherapeutic responses than 2D monolayers.
- 2) Gynaecological malignancies will exhibit a distinct reliance on D2-like receptor signalling; consequently, targeted antagonism will significantly impair cancer cell viability and migratory capacity.
- 3) Domperidone-DRD2 interactions at the extracellular cell surface will downregulate key tumorigenic DRD2 survival pathways.
- 4) DRD2 blockade via Domperidone will elicit anti-cancer and immunomodulatory activity in 3D OTSC gynaecological slices and demonstrate efficacy in patient-derived biopsy slices.
- 5) Encapsulating Domperidone within a PLGA-PEG polymeric carrier will overcome its inherent solubility and bioavailability limitations and facilitate cellular uptake and cytotoxicity in 2D monolayer and 3D OTSC models of gynaecological cancers.

Chapter 2

Development of OTSC Models of
Ovarian and Endometrial Cancer and
their Validation with Chemotherapy

As established in Section 1.3, traditional 2D monolayer cultures often fail to predict clinical outcomes due to their inability to recapitulate the 3D architecture and heterogeneous complexity of the tumour microenvironment. While advanced 3D platforms like organoids and bioprinted models offer improvements, they often rely on the artificial reassembly of components. In contrast, Organotypic Tumour Slice Culture (OTSC) preserves the native cellular spatiality, extracellular matrix cues, and signalling interactome of the original tissue *ex vivo*, offering a more physiologically relevant environment for drug discovery.

Despite the clear translational advantages of OTSC, its application in gynaecological oncology remains disproportionately low compared to other malignancies. To bridge this gap, this chapter details the development and characterisation of a high-precision OTSC protocol tailored for High-Grade Serous Ovarian Cancer (HGSOC) and low-grade Endometrial Cancer (EC).

Chapter Aims

The primary objective of this chapter was to establish a reproducible *ex vivo* pipeline using two distinct models: the OVCAR8 cell line (representing HGSOC) and the ISHIKAWA cell line (representing low-grade EC). Specifically, this chapter aims to:

- **Optimise Slicing and Maintenance:** Refine vibratome parameters and culture conditions to maintain tissue integrity over an 8-day period.
- **Characterise Viability and Population Stability:** Evaluate cellular proliferation, death, and population composition using real-time functional assays, immunohistochemistry (IHC) and flow cytometry.
- **Validate Chemotherapeutic Sensitivity:** Assess the model's utility as a drug-screening platform by evaluating the response to 'standard-of-care' agents, Carboplatin and Paclitaxel, through viability assays and biomarker expression analysis.

By establishing these validated OTSC protocols, this work provides a high-fidelity platform for exploring novel therapeutic strategies that target both the tumour bulk and the surrounding microenvironment.

2.1 Methods

2.1.1 Cell Culture

The OVCAR8 cell line (NCI-60, RRID: CVCL_1629), representing HGSOC was obtained from the National Cancer Institute (NCI), and ISHIKAWA (CVCL_2529) representing low-grade endometrial cancer was from the American Type Culture Collection (ATCC) (Table 12). OVCAR8 cells were cultured in RPMI-1640 media (RPMI, GIBCO) and ISHIKAWA cells in *Dulbecco's Modified Eagle Medium* high glucose (DMEM, GIBCO), both supplemented with 10% foetal bovine serum (FBS, GIBCO), 2mM glutamine, 100 µg/mL Penicillin-streptomycin, and 1 µg/mL Amphotericin B (Life Technologies). Cells were cultured as per ATCC and NCI guidelines and kept at 37°C in a humidified environment of 5% CO₂.

Table 12. HGSOC and EC cancer cell line characteristics.

Cell Line	Cancer	Age	Derivation	Gene Expression (Transcripts per million)					Mutational Profile (mt = mutated, wt = wildtype)				
				ER	HER2	HER3	EGFR	FRα	KRAS	TP53	PTEN	CTNNB1	PIK3R1
OVCAR8	HGSOC	64	Primary tumour	- (0.019)	+ (28.2)	++ (38.3)	++ (27.4)	+ (11.0)	mt	mt	wt	mt	wt
ISHIKAWA	Low-grade EC	39	Primary tumour	+ (16.4)	++ (66.5)	++ (40.7)	+ (11.6)	++ (58.2)	wt	mt	mt	wt	mt

2.1.2 Xenograft Implantation

For implantation, the cells were washed in PBS, trypsinised (0.05% trypsin-EDTA, GIBCO) and collected at 300×g. Approximately, 2×10^7 cells were subcutaneously inoculated into 2 flanks of anaesthetised female athymic nude mice (Envigo, Inotiv). Alternatively, previously dissected and frozen explants (~1mm³) were implanted into the two flanks using the trocar system. Tumours were grown and excised once they reached a diameter of 7-10 mm (volume: ~500 mm³) and immediately washed and transported in cold media.

2.1.3 Optimisation of Organotypic Tumour Slice Culture

The tumours were trimmed of any external mucinous material, fats and blood which can lead to interference with the vibratome blade during slicing. To develop the slicing protocol, the relevant parameters were taken into account: percentage of agarose, buffer composition, temperature of sample prior to cutting, as well as slicing speed, blade amplitude and blade frequency (Table 13). All parameters were adjusted according to the consistency and density of the tumour (see table).

Table 13. *Vibratome slicing parameters for OVCAR8 and ISHIKAWA tumours.*

Cell line	Agarose formula	Slice thickness	Blade angle	Speed	Freq.	Amp.	Media
OVCAR8	6% in PBS/1%Pen-Step	300uM	5°	4.5 (0.15 mm/s)	60 Hz	1.0 mm	RPMI 10% FBS
ISHIKAWA	6%/9% in PBS/1%Pen-Step	300uM	5°	1.0-3.5 (0.025- 0.15 mm/s)	80 Hz	1.0 mm	DMEM-High 10% FBS

For standardisation, the tumours were cut into cores with a 6 mm biopsy punch and placed in an embedding mould designed by our laboratory (Figure 13). Cores were covered with preheated (37°C) agarose (Sigma) in PBS/1% Pen-Strep within in a laminar flow cabinet.

Initially, tumour cores were embedded in standard 24-well plates; however, this approach presented significant technical hurdles. The primary issue was gravitational settling, where tissue cores would sink to the bottom of the well. This positioned the tumour too close to the cutting base, where the blade is programmed to stop cutting ~0.3 cm from the bottom, resulting in loss of viable tissue. Furthermore, softer tumours (e.g., ISHIKAWA) lacked the inherent density to resist the mechanical force of the vibratome blade, resulting in irregular slices. To overcome this, we implemented a dual-density embedding strategy using 6% agarose as the initial layer, and a high-percentage (9%) agarose as a support behind the tissue to provide mechanical resistance.

Execution of this strategy required a 90° rotation of the solidified block to align the tissue with the high-density support furthest away relative to the blade. Standard 24-well plates produce blocks with rounded edges that cannot be precisely rotated or securely

immobilized on a flat slicing disc. To resolve these geometric constraints, we developed a cubic embedding device. This design allowed for exact 90° orientation and ensured that the tissue cores—positioned centrally on their sides during the first stage of embedding—retained a protective agarose buffer from the mounting disc after rotation, effectively eliminating tissue wastage (See Figure 13).

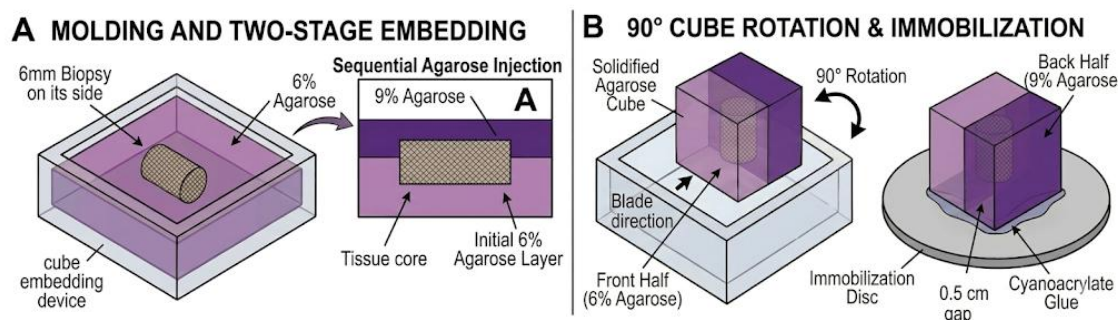


Figure 13. Diagram demonstrating the dual-density agarose embedding strategy within the custom-designed cube-shaped mould. (A) The tumour core is placed longitudinally in the embedding device covered with 6% agarose/PBS, ensuring gaps of ~0.5 cm are left either side of the core. Then, 9% agarose/PBS is added to function as a resistance support for softer tissues. **(B)** The solidified agarose block is turned 90 degrees and glued to the immobilisation disc with cyanoacrylate glue, facing the higher percentage agarose to the side furthest from the blade.

For OVCAR8 tumours, 6% agarose/PBS was used for embedding. For the softer ISHIKAWA tumours, the two-stage agarose process was employed using 6% and 9%, ensuring the lower percentage had partially solidified before adding the higher percentage. The specimen was left in the mould to stabilise at room temperature for ~5 min, then at 4°C for ~15 min, and finally kept on ice until use. The specimen was then immobilised to the disc with cyanoacrylate glue (Figure 14). Once fixed, the embedded specimen was placed in the slicing chamber with ice-cold PBS/1% Pen-strep and the core cut into identical circular slices.

After optimising the procedure, all tumours were cut at a slice size of 300 µm, blade angle of 5°, and amplitude of 1.0 mm with a Leica 1100s vibratome (Leica Biosystems). The slicing speeds and frequencies were adjusted according to the consistency and density of the tumour: for OVCAR8, the tumours sliced well at speeds of 3.5-4.5 (0.15 - 0.2 mm/s)

with a frequency of ~65 Hz, whereas the softer ISHIKAWA tumours required slightly slower speeds of 2.0-3.5 (0.075 - 0.15 mm/s) and higher frequencies (~80 Hz).

2.1.4 OTSC Cell Culture

Tumour slices were cultured in 24-well culture plates for up to 8 days in the same media used for their cell line constituents (1 mL media/well) and were kept in a humidified atmosphere of 37°C/5% CO₂. The slices were cultured using the ‘free floating’ method in which plates were constantly agitated using a plate shaker (Biosan, CPS-20) set to 70 RPM within the incubator. Media was changed every 1-2 days (Figure 14).

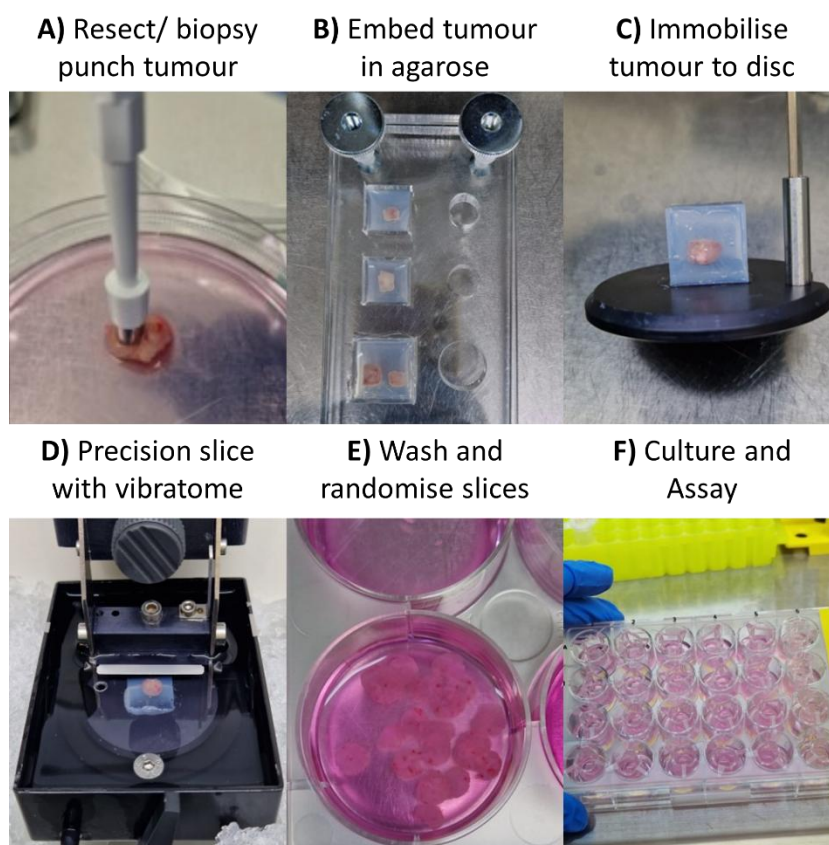


Figure 14. The Organotypic Tumour Slice Culture (OTSC) Workflow. (A) Uniform tissue cores are generated from the bulk tumour specimen using a biopsy punch. (B) Cores are immobilised within agarose blocks. (C) The embedded core is secured to the specimen disc using cyanoacrylate glue. (D) A high-precision vibratome is utilised to generate uniform, ultra-thin slices. (E) Slices are collected and randomised. (F) Individual slices are transferred to a multi-well plate and drug responses assayed.

2.1.5 Haematoxylin & Eosin (H&E)/ Immunohistochemistry

In order to assess OTSC slice viability during an 8-day culture period, slices were placed in cassettes and submerged in formaldehyde (3.7%) at fixed time points (Day 0, 1, 2, 4, and 8) in duplicate. Then, histological architecture, proliferation and cell death were assessed using H&E and appropriate biomarkers (ki-67, cleaved-caspase-3) as follows:

(a) Fixation and Dehydration: Prior to paraformaldehyde fixation, the slices were placed in HistoGel (Thermo Scientific) to stabilise them in a flat formation during paraffinization, ensuring efficient downstream sectioning by the microtome and then kept in a labelled cassette. Following a minimum of 24h in 3.7% formaldehyde, the HistoGel blocks were rinsed with distilled water and slowly dehydrated – a step necessary for the substitution of aqueous solutions for hydrophobic paraffin – by immersing the samples in increasing concentrations of ethanol (70, 80, 95 and 100%). To remove the alcohol, the samples were then incubated in xylene three times for 10 minutes.

(b) Paraffinization and sectioning: After dehydration, the sample is solidified in paraffin wax. The paraffin is first liquified by heating it to 60°C, whereby it can infiltrate the sample. The sample is then left to cool to 20°C and solidify. The sample is now stabilized, allowing thin slices of ~3-5 µm to be cut from the embedded tissue for immunohistochemistry assays. The sections were then mounted onto glass microscopy slides.

(c) Dewaxing and Rehydration: In order to be stained by H&E or immunohistochemical antibodies, the paraffin must be removed from the samples and rehydrated. Therefore, slides were left for 1h at 60°C, incubated twice in xylene, and gradually rehydrated using decreasing ethanol concentrations (100, 96, 80 and 50%).

(d) Hematoxylin & Eosin staining: The mounted and rehydrated samples were then washed for 5 min with distilled water and stained for 10 min with Mayer-hematoxylin solution. The samples were then washed for 15 min 2-3 times until the structures start to become recognizable, and then counterstained with 0.2% eosin for 1-2 min and washed to remove excess eosin. Samples were then dehydrated again in ethanol, rinsed in xylene and covered with mounting media and a coverslip (Toluene-Free Mounting Medium, Dako).

(e) Immunohistochemistry staining: To assess cytotoxic changes based on specific molecular biomarkers, freshly prepared microsections were stained with selectively targeting antibodies specific for cleaved-caspase-3 (c-Caspase-3; apoptosis) and ki67 (proliferation) biomarkers.

Tissue sections were mounted on positively charged slides (FLEX IHC Microscope Slides, Dako) to ensure optimal sample adhesion during processing. Sections were incubated at 60°C for 1 hour, followed by rehydration according to the protocol described in Section 2.5.3. Heat-induced epitope retrieval was performed using the PT Link system (Dako). The retrieval cycle consisted of an initial incubation for 10 minutes at 95°C and then 45 minutes at 65°C in Tris/EDTA buffer (pH 9, Dako). Automated immunostaining was subsequently conducted using the Autostainer Link 48 (Dako) and the EnVision Flex High pH kit (Dako). The programmed sequence included 5-minute incubation with Peroxidase-Blocking Reagent. Followed by 30-minute incubation with the specific primary antibody (see Table 14), 20-minute incubation with EnVision/HRP, Incubation with DAB+ Chromogen and Substrate Buffer, and counterstaining for 5-minute incubation with hematoxylin. Washes with rinse buffer for 1 minute were interspersed between steps.

Table 14. Antibodies used for IHC.

Antibody	Dilution	Source	Reference
Cleaved Caspase-3	1:300	BD Biosciences	560901
Ki67	1:200	Abcam	16667

2.1.6 Digital Quantification and Macro Scripting

Microscope slides were scanned and files analysed using the programme 3Dhistech Panoramic Pathology System (3DHISTECH Kft., Budapest, Hungary). Quantitative analysis of Ki67 and cleaved Caspase-3 staining was performed using ImageJ software. For each tumour slice sample, ten representative fields were captured at 400x magnification. To ensure reproducibility and minimise observer bias, a semi-supervised macro script was employed for automated quantification. This script facilitated the identification of positive immunostained areas, with final values expressed as the percentage of positive area relative to the total tumour area (Figure 15).

```

7 // edit the processFile() function at the end of this macro.
8 run("Set Measurements...", "area display redirect=None decimal=3");
9 requires("1.33s");
10 dir = getDirectory("Choose a Directory ");
11 setBatchMode(true);
12 count = 0;
13 countFiles(dir);
14 n = 0;
15 processFiles(dir);
16 //print(count+" files processed");
17
18 function countFiles(dir) {
19     list = getFileList(dir);
20     for (i=0; i<list.length; i++) {
21         if (endsWith(list[i], "/"))
22             countFiles(""+dir+list[i]);
23         else
24             count++;
25     }
26 }
27
28 function processFiles(dir) {
29     list = getFileList(dir);
30     for (i=0; i<list.length; i++) {
31         if (endsWith(list[i], "/"))
32             processFiles(""+dir+list[i]);
33         else {
34             showProgress(n++, count);
35             path = dir+list[i];
36             processFile(path);
37         }
38     }
39 }
40
41 function processFile(path) {
42     if (endsWith(path, ".tif")) {
43         open(path);
44
45         run("Colour Deconvolution", "vectors=[H DAB]");
46         close();
47
48         setOption("BlackBackground", false);
49         //run("Threshold...");
50         setThreshold(0, 40);
51         //setThreshold(0, 90);
52         setOption("BlackBackground", false);
53         run("Convert to Mask");
54         run("Create Selection");
55
56         run("Measure");
57         getValue("Area")
58             close();
59     }
60 }
61

```

Figure 15. Image J script for semi-automatised quantification of IHC staining. Each DAB image is separated by colour deconvolution, the appropriate threshold is set and the staining area is quantified.

2.1.7 IHC of Slices Treated with Chemotherapy

For IHC, the slices used for AlamarBlue Chemotherapy assessments were subsequently fixed in paraformaldehyde stained for proliferation (Ki67) and apoptosis biomarkers (IHC) and analysed as described in Section 2.1.5.

2.1.8 Viability Assessment (AlamarBlue)

In order to assess viability of the same slice in real-time during an 8-day culture period, the functional metabolic dye, AlamarBlue (Invitrogen) was utilised. AB is a non-toxic resazurin viability dye which quantitatively detects oxidation during cellular respiration. When in the presence of metabolically active cells, the dye is reduced from a blue to a pink fluorescently coloured solution. The more metabolically active cells present, the greater the fluorescence measured. Therefore, fluorescence can be measured over the course of culture or before and after therapeutic administration in the same slice, to quantitatively measure changes in slice health.

Firstly, Alamarblue (AB) was prepared at a 1:10 dilution in the appropriate media. Then, 1mL was added to the slices and incubated for 4h whilst rotating at 70 RPM under standard culture conditions. The media was then collected and 100 µl of each sample and background control (AB with no slice) were pipetted in triplicate in a 96-well plate. The fluorescence was immediately measured at 560/590 nm (excitation/emission) using the Infinite M200 Pro plate reader (Tecan). For 8-day viability assessments, this was conducted at the same time each day on Day 0, 1, 2, 3, 4, and 8. Relative viability was calculated using the following formula:

$$(A) RFU_{Corrected} = Mean RFU_{Experimental} - Mean RFU_{Background} \quad (1)$$

$$(B) Relative Day X (\%) = \frac{Mean RFU_{Corrected Day X}}{Mean RFU_{Corrected Day 0}} \times 100 \quad (2)$$

Where $RFU_{Corrected}$ represents the experimental fluorescence units after subtracting the mean background signal (media/AlamarBlue alone) measured at the corresponding time point.

2.1.9 Cytotoxicity Monitoring (LDH Cytotoxicity Assay)

To quantify cell death in the same slice during an 8-day culture period, the CyQUANT LDH assay (ThermoFisher) was used according to Manufacturer's instructions and following a similar protocol to Mckenna *et al.* (2021).

The media was collected every 24 hours e.g., Day 1, 2, 3, 4 and on Day 8 and kept in the fridge for no longer than 48h prior to testing, as samples kept for longer periods of time or frozen at -20°C resulted in signal loss. At the end time point, the slices were treated with 1% Triton X-100 (in media) for 2 hours and the supernatant collected again, in order to fully lyse any remaining cells in the slice and measure the remaining LDH.

For the assay, 50 µL of sample was pipetted into a 96 well plate, followed by 50 µL reaction mixture and incubated in the dark for 10 minutes. Then, 50 µL of stop solution is added and the plate read at 560/590nm. A media only control sample (no slice) was used to calculate background LDH that is sometimes found in media supplemented with 10% FBS. The fluorescence of the media-only sample is then subtracted from the experimental samples:

$$RFU_{Corrected} = Mean\ RFU_{Experimental} - Mean\ RFU_{media} \quad (3)$$

Using the sum of the fluorescent readings from each day and end time point with 1% Triton X-100, the total LDH in each slice could be calculated:

$$LDH_{Sum} = \sum_1^n RFU_{Corrected} \quad (4)$$

Where n = number of days.

Daily cell death was then expressed as a percentage of total LDH release:

$$LDH\ Release\ Day\ X\ (\%) = \frac{RFU_{Corrected\ Day\ X}}{LDH_{Sum}} \times 100 \quad (5)$$

Then, relative cytotoxicity was calculated using the following formula:

$$Relative\ Day_x\ (\%) = \frac{LDH\ Release\ Day\ X}{LDH\ Release\ Day\ 0} \times 100 \quad (6)$$

2.1.10 Cell Population Composition Analysis

To analyse population composition within the tumour and OTSC slices, the tissue was disaggregated and analysed by flow cytometry using fluorescent antibodies specific for population biomarkers. All reagents and equipment are from Miltenyi Biotec (Spain) unless otherwise stated.

2.1.11 Tumour Dissociation: Enzymes H, R, and A (Miltenyi Biotec Tumour Dissociation Kit) were reconstituted according to the manufacturer's instructions. Briefly, Enzyme H was dissolved in 3 mL of RPMI 1640 or DMEM, Enzyme R in 2.7 mL of the same medium, and Enzyme A in 1 mL of the supplied Buffer A. All solutions were aliquoted to avoid freeze-thaw cycles and stored at $-20\ ^\circ\text{C}$ until use. Tumour tissue was excised, trimmed of necrotic regions, and cut into 2–4 mm fragments before weighing. Tissue fragments were transferred into gentleMACS™ C Tubes containing enzyme mix prepared in RPMI 1640 or DMEM at ratios adjusted to tumour mass (e.g., for (0.05–1.0 g: 2.2 mL medium, 100 μL Enzyme H, 50 μL Enzyme R, 12.5 μL Enzyme A).

Dissociation was performed using the gentleMACS™ Octo Dissociator, selecting the programme according to tumour type (for ovarian and endometrial tumours 37C_h_TDK_1 for soft tumours was selected). After dissociation, samples were filtered through a 70 μm MACS SmartStrainer, washed twice with 5 mL medium and then 10 mL media, and centrifuged at $300 \times g$ for 7 min at room temperature. Erythrocyte lysis and DNase treatment (200 U/mL, 5 min) were performed when necessary. To enhance accuracy of downstream analyses, dead cells were removed with magnetic beads. Cell suspensions were processed with the Dead Cell Removal Kit (Miltenyi Biotec #130-090-101) following the manufacturer's protocol using the autoMACS Pro Separator. The resulting viable cell fraction was centrifuged, resuspended in PBS (100–200 μL), and used for downstream analyses.

2.1.12 Flow Cytometry (Tumour): The cell suspensions were incubated for 20 min in the dark using the antibodies and concentrations specified in Table 15. Cell suspensions were then washed twice in PBS and resuspended in 500 μ L PBS. Cell viability was determined using propidium iodide (PI) exclusion. A 1:100 PI stock solution was prepared in ddH₂O, and cells resuspended in PBS or PEB buffer were stained at a final dilution of 1:10,000. Samples were incubated in the dark at room temperature for 5 min, and 50 μ L was analysed with a MACSQuant® Analyzer 10 flow cytometer.

Table 15. Antibodies used for flow cytometry of cell populations.

Population	Marker	Dilution	Clone	Fluorochrome	Reference
Epithelial cells	EpCAM	1:50	HEA-125	PE-Vio 770	130-113-265
Pan-leukocyte	CD45	1:50	REA737	VioBlue®	130-110-802
Endothelial Cells	CD31	1:50	REA784	Vio-bright 515	130-111-544

2.1.13 Tumour Dissociation of OTSC Slices: OTSC slices are more fragile and easier to disaggregate than the original tumour bulk, therefore require a gentler protocol. Thus, to improve viability of cells during OTSC slice disaggregation, a gentler enzyme mix was used. Briefly, 3–4 slices per tumour were washed in PBS and enzymatically dissociated using TL Liberase (0.2 mg/mL; Roche) and DNase I (10 μ g/mL; Roche) in serum-free DMEM using a gentleMACS Dissociator (programme 37C_h_TDK_1, 1 h at 37 °C). Cell suspensions were filtered through 40 μ m strainers, washed with complete medium containing 10% FBS, and centrifuged at 300 g for 5 min at 4 °C. Pellets were resuspended in PBS with 2% FBS, and to calculate viable cells, a sample was quantified by Trypan Blue exclusion (Gibco).

2.1.14 Flow Cytometry (OTSC): For flow cytometry, cells were stained with a fixable viability dye (Miltenyi Viability™ 405/520) for 15 min in the dark at 4°C, washed twice with 1mL PBS/2% FBS, centrifuged at 300 g, 5 min at 4 °C followed by Fc receptor blocking (anti-CD16/CD32; BD biosciences #553142) for 15 min at 4°C. Without washing, the cell suspensions were then stained with antibody cocktails in Table 15 to identify epithelial, endothelial, and immune cells (EpCAM+, CD31+, CD45+). Staining was performed for 20 min at 4 °C in the dark, with single-colour compensation controls prepared in parallel. After staining, cells were washed, fixed in 4% paraformaldehyde for 20 min at room

temperature, resuspended in PBS/2% FBS, and stored at 4 °C in the dark until acquisition (within 1–2 days) on MACSQuant® Analyzer 10.

2.1.15 Validation with Chemotherapy

For drug assessments using the ‘standard of care’, 5 μ M Taxol and 300 μ M Carboplatin (Tocris Biosciences) were diluted in media and added to each well and left to incubate for 48 hrs rotating at 70 RPM under standard culture conditions. These concentrations were chosen as they correspond to previously used preclinical and clinical dosages. For example, for paclitaxel, 175 mg/m² at 3-hour infusion cycles (C_{max} = 5.1 μ M) is used to treat ovarian cancer patients (Stage et al, 2021). In the case of the carboplatin, a clinical concentration with an AUC of 5 (C_{max} = 149.2 μ M) has been reported, and in literature previous studies have tested concentrations as high as 300 μ M in the laboratory (Brodeur et al. 2021; Burkhard et al. 2025).

2.1.16 AlamarBlue and LDH Assays in Cell Lines with Chemotherapy

To assess 2D monolayer cell line drug responses, OVCAR8 and ISHIKAWA cell lines were seed at a density of 1×10^5 cells/mL in 0.5mL of appropriate media in 24-well plates. Cells were grown for 24h to reach a ~60-70% confluency, media removed and replaced with plain media (Control) or media containing 5 μ M Taxol and 300 μ M Carboplatin. Cells were incubated for 48h in standard culture conditions. On Day 3, AB dye (1:10) was added for 2h and fluorescence read in a 96-well plate. Difference in viability was quantified relative to Control wells. Whereas for LDH assessments, in a separate plate, media was collected prior to drug administration (Day 1) and 24h (Day 2) and 48h (Day 3) after drug administration. Cells were then treated with Triton X-100 (1%) for 2h and media collected, representing remaining intracellular LDH. LDH Release was quantified using the CyQUANT LDH assay as described in Section 2.1.9 and calculated relative to Day 1. The difference between Treated and Control LDH release at the end of the experiment was then calculated.

2.1.17 AlamarBlue and LDH Assays in Slices with Chemotherapy

To quantify the baseline viability of the slices prior to vehicle/chemotherapy administration, the slices were measured with 1:10 AlamarBlue dye on Day 0 and Day 1, as described above. Slices were then treated with 1mL 5 μ M Taxol/300 μ M Carboplatin or vehicle-only control in duplicate for 48h, rotating at 70 RPM and in standard culture conditions. AlamarBlue (1:10) was then incubated for 4h again on Day 3 and change in viability was quantified relative to Day 0 (Equations 1-2).

To calculate the difference between the treated and the vehicle-only control after 48h incubation (Day 3), the percentage change between conditions was calculated using the Day 3 AlamarBlue measurements normalised to Day 0 of the treated slices relative to the matched control slices:

$$\begin{aligned} & \text{Difference between Treated and Control Slices (\%)} \\ &= \frac{Treated_{Relative\ Day\ 3}}{Control_{Relative\ Day\ 3}} \times 100 \end{aligned} \quad (7)$$

For changes in cell death, media from 2 slices per condition were collected after 24h in culture prior to drug administration (Day 1), and every 24h after drug/vehicle administration (e.g., Day 2 and 3) and quantified using the CyQUANT LDH assay as described in Section 2.1.9 (Equations 4-7).

2.1.18 Statistical Analysis

To assess the effect of culture or chemotherapy treatment over time on viability and cytotoxicity, Linear Mixed-Effects Models (LMM) were performed in R (v.4.1.2) using the lme4, car, and emmeans packages, with treatment group as a fixed effect and Slice ID included as a random effect to account for the paired nature between control and treated slices. Normality of model residuals was assessed using the Shapiro-Wilk test and Q-Q plots. If residuals were not normally distributed, $\log_{10}$ and square root transformations were applied. In cases where data could not be normally distributed with transformations (e.g., the LDH data), Generalized Linear Mixed Model (GLMM) with a Gamma distribution and log-link function were used. For data normalised to a control baseline (set to 100%), One-Sample t-tests were performed to compare the treatment

group means against the theoretical value of 100. Normality of the data distributions was confirmed using the Shapiro-Wilk test. For non-normal normalised data (LDH), a One-Sample Wilcoxon Signed Rank test was employed to determine if the median significantly deviated from the theoretical value of 100. For the comparison of culture models (2D monolayers vs. 3D slices), unpaired Welch's t-tests were used to account for unequal variances between the two distinct culture conditions.

To evaluate the overall differences between whole tumours and cultured slices across multiple cell populations, an LMM was constructed as before, with treatment group and cell marker as fixed effects and Slice ID as a random effect. To analyse differences of individual markers between xenografted models (OVCAR8 vs. ISHIKAWA), unpaired Welch's t-tests were performed due to unequal variances and varying sample sizes. All statistical analyses were performed using R, and a p-value of < 0.05 was considered statistically significant.

2.2 Results

2.2.1 OVCAR8 and ISHIKAWA Slices Retain Metabolic Viability up to Day 4 in Culture

In order to assess the viability of the OTSC slices, the metabolic activity and LDH release from the same slice was measured periodically up to Day 8. For viability, AlamarBlue dye was incubated with the same slice for 4h on Day 0, 1, 2, 3, 4 and 8, and the media collected and measured by fluorescence. For LDH, a sample of the media from Day 1, 2, 3, 4, 6, and 8 of the same slice was collected and quantitatively measured using a commercial kit. To support real-time metabolic assay measurements, a fresh slice was fixed on Day 0, 1, 2, 4, and 8 and analysed for proliferation (ki67) and apoptotic cell death (cleaved-caspase-3) by IHC and change in area of staining during culture was quantified.

For OVCAR8 slices, real-time metabolic assays showed the OTSC slices were stable between Day 1 and Day 4, with AlamarBlue viability measurements ranging from 92.3-101.5% compared to Day 1 (baseline = 100%). However, there was a significant loss of viability by Day 8 of 33.4% ($p < 0.0001$) (Figure 16A). Similarly, immunohistochemistry of fixed slices using Ki67 staining showed proliferation steadily reducing from baseline (100%) to 81.7% by Day 1 ($p = 0.0162$), 71.3% Day 2 ($p = 0.0023$), 63.7% Day 4 ($p = 0.0003$), and 35.6% by Day 8 ($p < 0.0001$). However, only Day 8 was significantly different from Day 1 ($p < 0.0001$), indicating reasonable stability between Day 1 and 4 (Figure 16C).

As seen in Figure 16B, cell death assessments also indicated slice health, with LDH release steadily decreasing between Day 1 and 3 (80.3-75.2%) reaching a significant reduction of LDH release of 57.2% by Day 4 ($p = 0.0171$) and 34.6% Day 6 ($p = 0.0004$), however by Day 8 there was a significant increase in LDH release from the slice with an average of to 146% compared to baseline ($p = 0.0094$). Moreover, apoptotic cell death measured by cleaved-Caspase-3 staining also remained highly stable. Although staining appeared to gradually increase after Day 1 (98%) with values of 102% (Day 2) and 116% (Day 4) before falling again to 93.4% by Day 8, these differences were not statistically significant (Figure 16D-E).

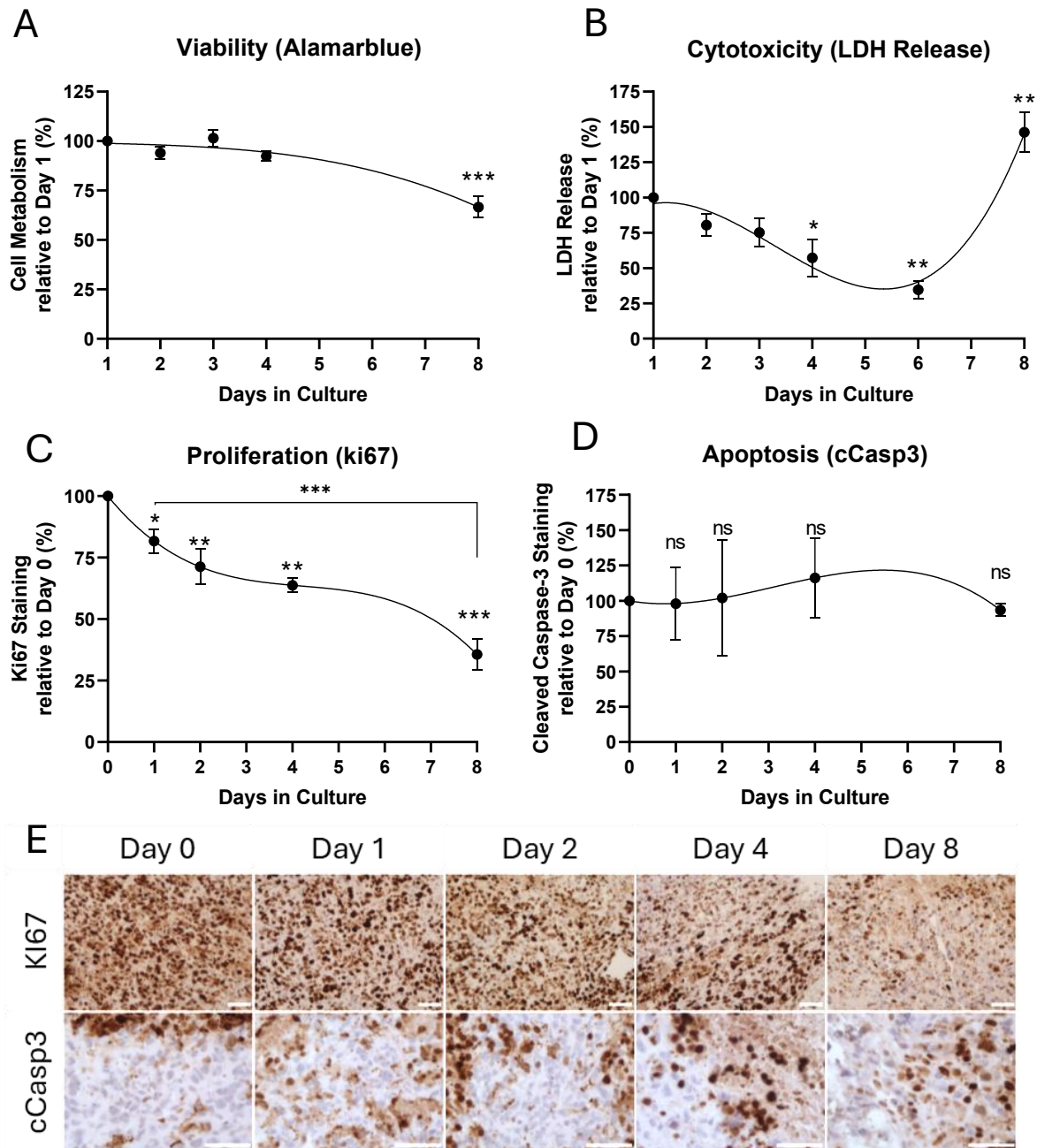


Figure 16. **A)** Viability and **B)** cytotoxicity measured by changes in AlamarBlue metabolism ($n=8$) and lactate dehydrogenase (LDH) production ($n=5$) in ovarian (OVCAR8) OTSC slices, respectively, relative to Day 1. **C)** Cell proliferation and **D)** Apoptotic cell death measured by changes in ki67 and cleaved-caspase-3 IHC staining, respectively, relative to Day 0 ($n=3$) with **E)** Representative microscopy images below. Graphs plotted using mean $\pm$ SEM. Linear mixed modelling was used to detect statistical significance, ns = not significant, $*p<0.05$, $**p<0.01$, $***p<0.0001$. Scale bar = 50 μ m.

For ISHIKAWA slices, real-time metabolic assays showed the OTSC slices were less stable than OVCAR8 OTSC slices and after day 1 had significantly decreasing viability compared to Day 0 (100%), with values of 76.1% (Day 2; $p = 0.008$), 67.2% (Day 3; $p = 0.0003$), 59.1% (Day 4; $p < 0.0001$), and 37.5% by Day 8 ($p < 0.0001$) (Figure 17A). However, compared to Day 1, only Day 4 ($p = 0.0045$) and Day 8 ($p < 0.0001$) were significantly different to Day 1, indicating stability between Day 1 and Day 3. In contrast, IHC proliferation Ki67 staining remained relatively stable between Day 0 and Day 4 (100% to 72.5%), however proliferation levels were significantly reduced by Day 8 with a value of 41.1% ($p = 0.0066$) (Figure 17C).

While cell death initially displayed a reduction, with the lowest level of LDH release being recorded on Day 2 (63.3%), LDH release then began to rise to 104% on Day 3 and then to 126.9% by Day 4 (Figure 17B). After this height, LDH release reduced slightly on Days 6 and 8 (118.6 and 106%). However, LDH release measurements were not statistically different from Day 0, with the only statistical significance being achieved between the lowest value on Day 2 and the highest value on Day 4 ($p = 0.0114$) (Figure 17). Cleaved-caspase-3 staining followed a similar pattern with a reduction on Day 1 and 2 (65.2%-79.8%) and a large increase on Day 4 (182.2%). By Day 8, very minimal staining was observed (20.9%), however there were also large patches of tissue with no cells present. While variability between days was observed, no statistically significant differences were recorded (Figure 17D-E).

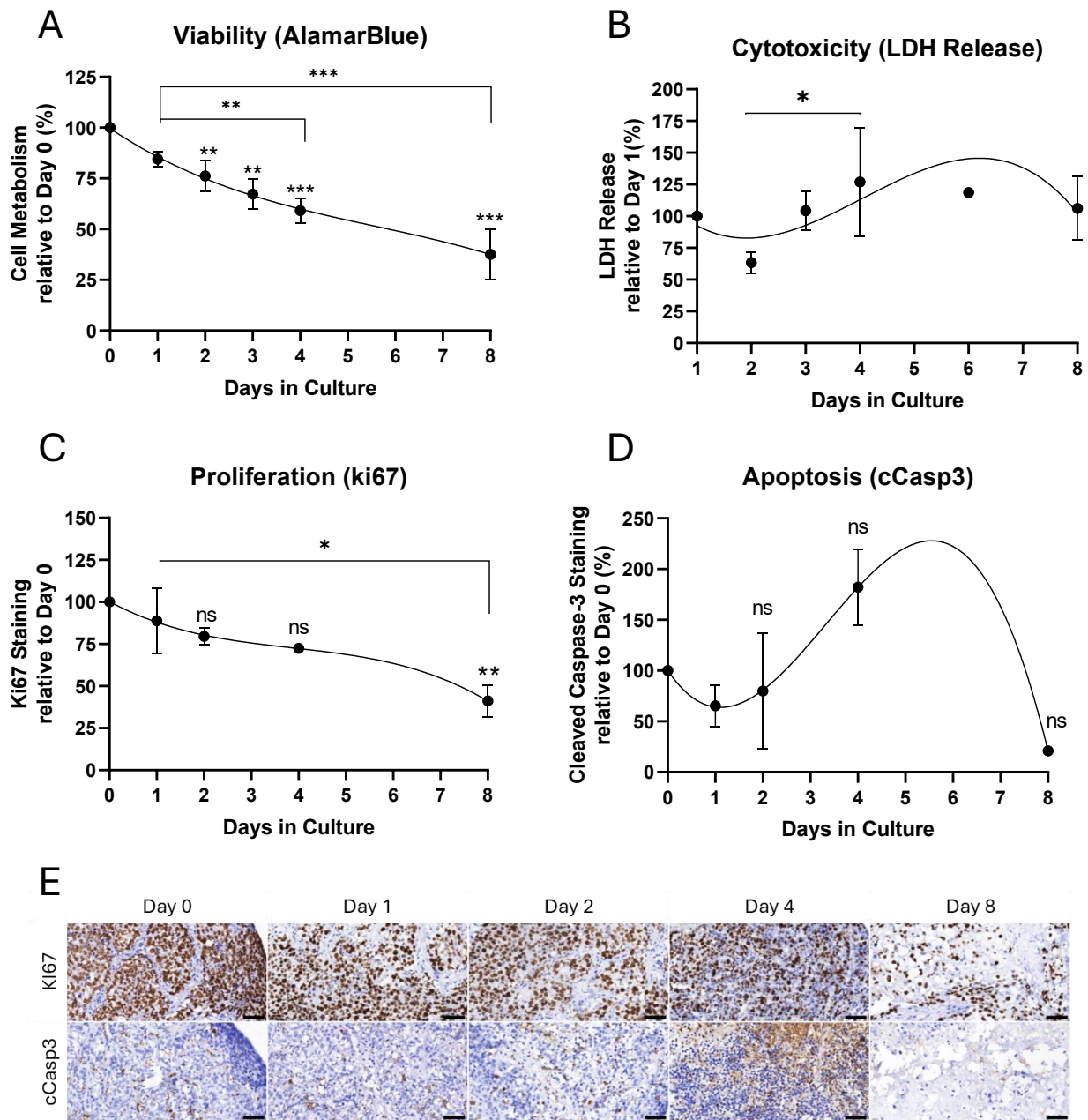


Figure 17. A) Viability and B) cytotoxicity in endometrial (ISHIKAWA) OTSC slices measured by changes in AlamarBlue metabolism ($n=9$) and lactate dehydrogenase (LDH) production ($n=8$), respectively, relative to Day 0 or Day 1. **C) Cell proliferation and D) Apoptotic cell death** measured by changes in ki67 and cleaved-caspase-3 IHC staining, respectively, relative to Day 0 ($n=4$) with **E) Representative microscopy images** below. Graphs plotted using mean $\pm$ SEM. Linear mixed modelling was used to detect statistical significance, ns = not significant, $*p<0.05$, $**p<0.01$, $***p<0.0001$. Scale bar = 50 μ m.

2.2.2 OTSC Platform Successfully Recapitulates Native TME Heterogeneity

To confirm the presence of heterogenic cell populations and assess their abundance in whole xenograft tumours, tumours were explanted and immediately disaggregated by mechanical and enzymatic digestion. Flow cytometry was then employed to assess the presence of cancer epithelial cells (EpCAM+), immune cells (CD45+) and endothelial cells (CD31+).

Both OVCAR8 and ISHIKAWA whole disaggregated tumours had similar levels of CD45+ immune cells with values of 18.2% and 15.5% ($t(4.3)=-0.686$, $p=0.528$), respectively (Figure 18A). While ISHIKAWA tumours had more EpCAM+ epithelial cells (81.2%) than OVCAR8 (70.9%), this 10.3% difference did not reach statistical significance ($t(9)=1.447$, $p=0.182$). In contrast, OVCAR8 tumours had significantly more CD31+ endothelial cells than ISHIKAWA tumours with values of 0.7% and 0.3%, respectively ($t(12.8)=-2.537$, $p=0.025$), alongside a greater portion of uncategorised cells (12.9% vs. 2.1%) (Figure 18A).

For OTSC slices, we wanted to assess the populations within the tissue following slicing and culture, therefore slices were cultured for 48h in media prior to disaggregation. When comparing the cultured slices and the original tumour mass, ISHIKAWA slices had relatively similar proportion of EpCAM+ cells to the tumour mass, with a value of 81.3% ($t(16.9)=0.021$, $p=0.984$) (Figure 18B). While slices experienced on average a ~50% decrease in CD45+ cells (8.4% vs. 16.4%), this difference was not statistically significant ($t(17)=-0.250$, $p=0.806$). However, ISHIKAWA slices had a significant 7-fold increase in endothelial cells compared to the whole disaggregated tumours ($t(16.9)=4.303$, $p=0.0005$). Similarly, OVCAR8 cultured slices had a minimal non-significant loss of CD45+ cells ($t(32.4)=0.093$, $p=0.927$), however EPCAM+ cells were reduced by approximately 50% ($t(32.4)=-2.920$, $p=0.0063$). Moreover, endothelial CD31+ cells also decreased by ~50%, however this did not reach statistical significance ($t(32.4)=-1.046$, $p=0.303$) (Figure 18B).

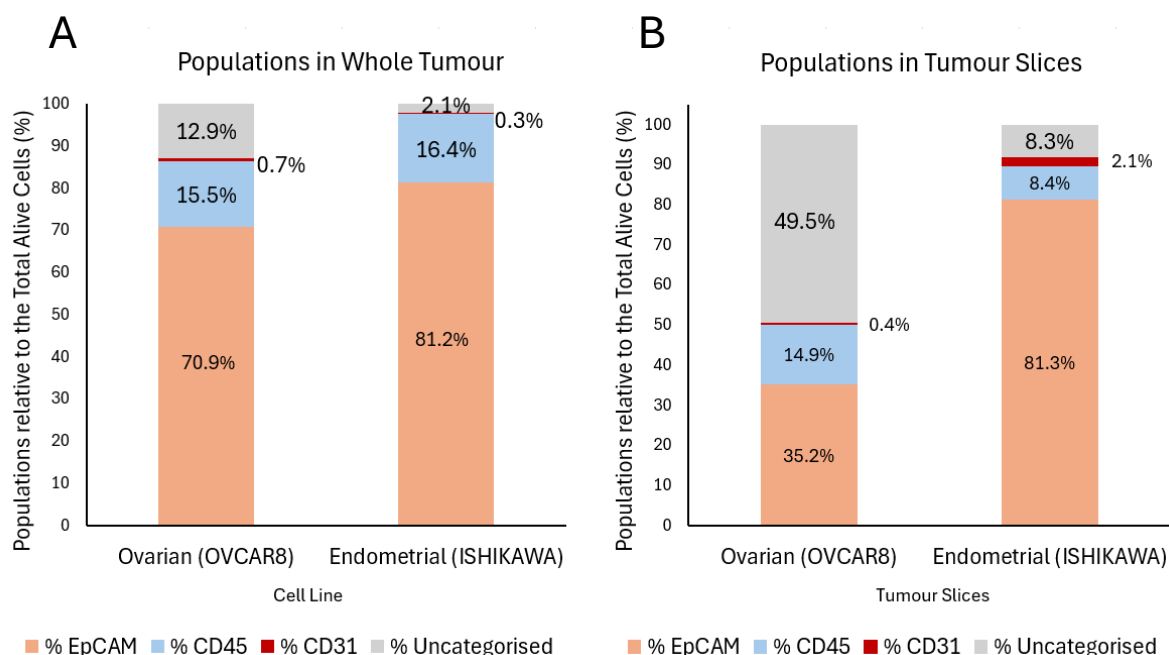


Figure 18. Cell Populations in **(A)** whole disaggregated tumours and **(B)** disaggregated tumour slices derived from OVCAR8 or ISHIKAWA xenografted tumour. Presented as percentages relative to total alive cells using biomarkers of epithelial cells (anti-EpCAM; orange), immune cells (anti-CD45; blue), endothelial cells (anti-CD31; red) and uncategorised cells (grey). Plotted as mean % (ovarian $n=3-8$, endometrial $n=3-4$).

2.2.3 OVCAR8 Slices Exhibit Cytotoxicity to Chemotherapy

To validate our ovarian cancer OTSC model, we needed to test the approved standard-of-care (5 μ M Taxol/300 μ M Carboplatin) in both the original OVCAR8 cell lines (2D) and in the cell line-derived xenografted OTSC slices (3D). The OVCAR8 OTSC slices were cultured for 24h prior to drug administration to allow the slice to recover from slicing. Then 5 μ M Taxol/300 μ M Carboplatin was incubated for 48h, and changes in viability and cell death using the real-time and fixed assays were evaluated. AlamarBlue dye was used on Day 0 and 1 to record the slice health at baseline, and then 48h later on Day 3 to measure viability after therapy. Media was also collected from separate slices on Day 1 (baseline) and after drug administration on Day 3 to assess real-time changes in cell damage via LDH release. To complement these functional assays, the AlamarBlue slices were either fixed and tested via IHC for cytotoxicity (Figure 19A-G).

Results show that OVCAR8 control slices maintained good slice health throughout culture with an initial increase of 22% in viability between Day 0 and 1, and they maintained this level until Day 3 (120.2%) (Figure 19A). The chemotherapy-treated slices also had a slight increase in viability on Day 1, however following drug administration, slice health significantly declined to 37.6%. Comparing the treated and control slices, metabolic activity at Day 1 revealed no significant difference between groups prior to treatment ($p = 0.273$), whereas following 48h incubation with chemotherapy, treated slices were significantly less viable ($p = 0.017$) (Figure 19A-B). However, analysis of the proliferation marker Ki67 revealed high variation between the three samples (Figure 19C). While one sample achieved a 46% reduction in proliferative staining, the other two samples ranged from ~5-10% reduction, achieving an average viability of 83.5% for the treated condition compared to the control (100%). This 17% loss of viability was not significantly different in one sample T-test analysis ($p = 0.225$). However, it should be noted that sample size was very small ($n = 3$). Interestingly, the 2D cell line grown in a monolayer format was significantly more sensitive to chemotherapy than the 3D slices ($p = 0.02$). The 2D OVCAR8 cells displayed 3.8% viability compared to the control wells (100%), whereas the 3D slices had a viability of 38.6% compared to the control slices (100%) (Figure 19F).

Cytotoxicity assays also showed differences in LDH release in the control and treated slices (19.9% vs. 26.3%) however this increase did not reach significance ($p = 0.250$) (Figure 19D). In contrast, analysis of cleaved Caspase-3 staining revealed marked 326-fold increase in the cell death marker, reaching the statistical significance threshold in one sample T-test analysis ($p = 0.0143$) (Figure 19E). While the 2D cell line showed higher cytotoxicity to chemotherapy, with an LDH release of 222.5% compared to 3D slices (137.7%), this increase did not reach statistical significance ($p = 0.169$) (Figure 19G).

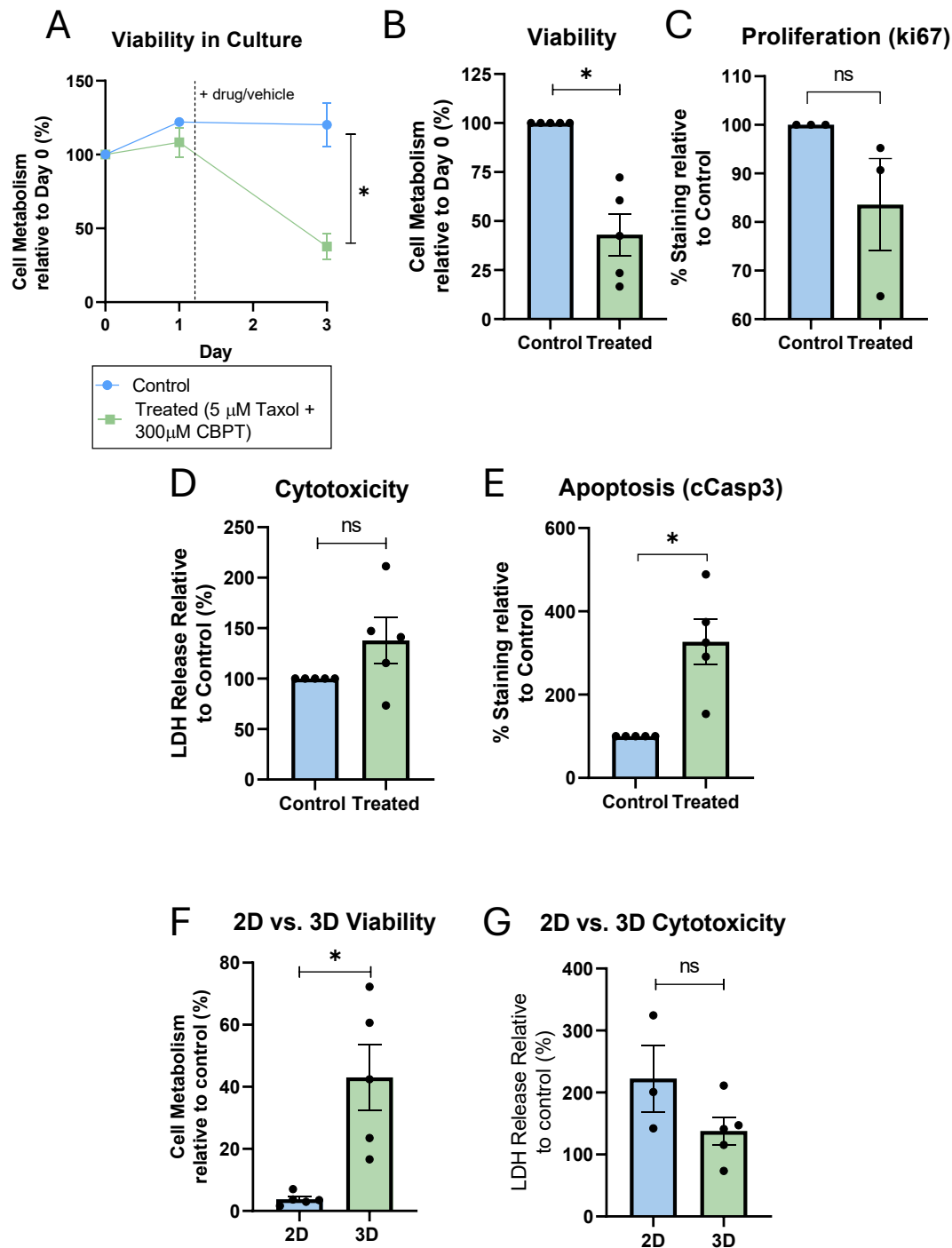


Figure 19. (A) Metabolic activity of OVCAR8 slices over a 3-day culture period. Taxol/carboplatin (5 μ M/300 μ M) or vehicle were administered for 48h after 24h in culture (dotted line). (B) Metabolic activity on Day 3 normalised to control (n = 5). (C) Proliferation (Ki67) IHC staining intensity relative to control (n = 3). (D) Cell death (LDH release) on Day 3 relative to control (%), n = 5. (E) Apoptosis (cleaved-Caspase 3; cCasp3) IHC staining relative to control (n = 4). (F-G) Comparison of 2D (cultured cells) versus 3D (tumour slices) systems treated with chemotherapy normalised to control: (F) Cell viability (AlamarBlue; n = 5). (G) Cytotoxicity (LDH release) (n = 3–5). All data presented as mean $\pm$ SEM, performed in duplicate. Tested with Linear Mixed-Effects Model (A), One-Sample t-test (B-E), and Welch's t-test (F-G), *p < 0.05, ns = not significant.

2.2.4 ISHIKAWA Slices Exhibit Resistance to Standard Chemotherapy

Like with OVCAR8 OTSC slices, ISHIKAWA slices were cultured and 5 μ M Taxol/300 μ M Carboplatin administered for 48h. Changes in viability and cell death were assessed using AlamarBlue dye on Day 0, 1 and 3, and LDH release measured from separate slices on Day 1 (baseline) and after drug administration on Day 2 and Day 3, complemented by IHC (Figure 20A-H).

Results show that ISHIKAWA OTSC control and treated slices exhibited a decline in slice health of ~20% between Day 0 and 1, and they maintained this level until Day 3 with control reaching 76.3% viability and the treated slices with a slightly higher mean value of 84.5%, compared to their own individual baselines (100%) (Figure 20A). Between groups, statistical analysis revealed that mean metabolic activity was not significantly different at either Day 1 (LMM, $p = 0.721$) or Day 3 ($p = 0.391$) (Figure 20A). Similarly, data normalised to control slices revealed the 113% viability of the treated slices did not reach statistical significance (One-sample T-test, $p=0.176$) (Figure 20B). Quantitative analysis of Ki67 staining (proliferation) revealed that chemotherapy treatment reduced the proliferative staining to 87.0% of the control baseline (100%) (Figure 20C). However, analysis indicated that this reduction was not statistically significant ($p = 0.686$). Interestingly, the ISHIKAWA cell line grown in a 2D monolayer format was significantly more sensitive to chemotherapy than 3D slices, yielding a viability of only 15% (Welch's Test, $p < 0.0001$) (Figure 20G).

In contrast, cytotoxicity assays showed lower LDH release in the control and treated slices (97% and 70.7%) after 24h drug administration (Day 2), which were not significantly different between groups ($p=0.361$). However, by Day 3 both control and treated slices had an increase in LDH release, with the treated slices maintaining a significantly higher cytotoxicity signal of 211% compared to the control mean of 151.1% (GLMM, $p=0.0023$). This result was confirmed by the control-normalised data, exhibiting an average 84.9% increase in cytotoxicity in the treated slices (One-sample Wilcoxon, $p= 0.06$) (Figure 20D-E). While this result was just shy of significance ($p > 0.05$), it should be noted that due to the small sample size ($n=5$) and the nature of the non-parametric Wilcoxon test, the smallest p-value possible of this sample size is 0.06. Furthermore, analysis of cleaved-Caspase 3 (apoptosis) revealed a massive and highly significant induction of cell death,

with chemotherapy-treated slices exhibiting a dramatic increase in apoptotic marker expression, reaching 836.4% relative to the control baseline (100%) (Figure 20F). Statistical analysis confirmed this increase was highly significant ($p = 0.007$). Similarly, 2D cell lines also had a slightly greater level of cytotoxicity than 3D slices (151.1% vs. 134.4%) but this failed to reach statistical significance (Welch's Test, $p = 0.125$) (Figure 20H).

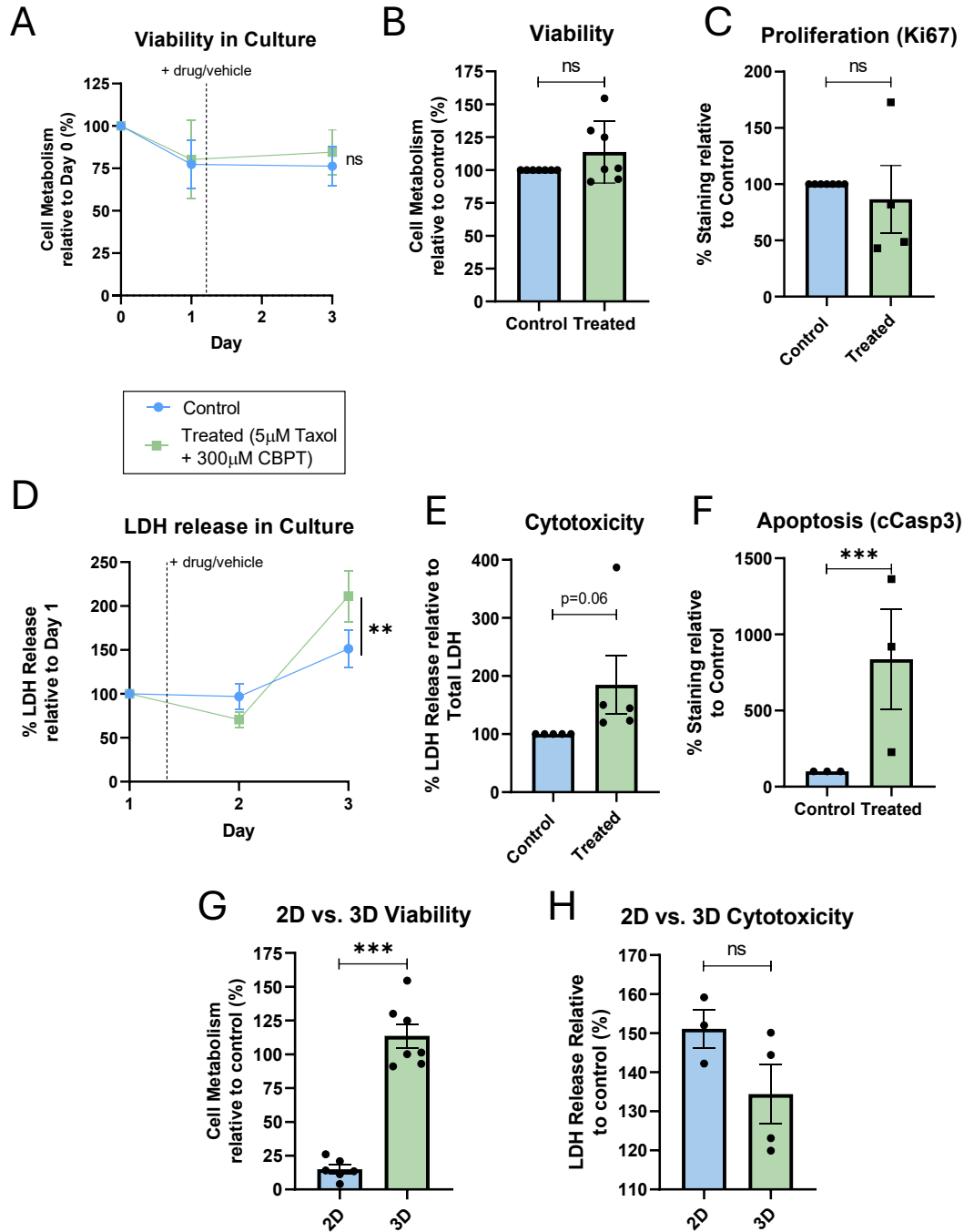


Figure 20. (A) Metabolic activity (AlamarBlue) of ISHIIKAWA slices over a 3-day culture period ($n = 3$). Taxol/carboplatin (5 μ M/300 μ M) or vehicle were administered for 48h after 24h in culture (dotted line). (B) Metabolic activity on Day 3 normalised to control ($n = 7$). (C) Proliferation (Ki67) IHC staining intensity relative to control ($n = 4$). (D) Cell death (LDH release) in culture ($n = 5$). (E) Cytotoxicity (LDH release) on Day 3 normalised to control ($n = 5$). (F) Apoptosis (cleaved-Caspase 3; cCasp3) IHC staining relative to control ($n = 4$). (G-H) Comparison of 2D (cells) and 3D (tumour slices) treated with chemotherapy: (G) Cell viability (AlamarBlue, $n = 6-7$). (H) LDH release ($n = 3-4$). All data presented as mean $\pm$ SEM, performed in duplicate. Tested with Linear Mixed-Effects Model (A), One-Sample t -test (B-C, F), generalised linear mixed modelling (D), Wilcoxon Signed Rank (E), and Welch's t -test (G-H), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, ns = not significant.

Chapter 3

Characterising the Dopaminergic
Interactome in Ovarian and Endometrial
Cancer Cell Lines and the Screening of
Dopaminergic Modulators

In gynaecological oncology, aberrant DR expression in tumour biopsies has been linked to poorer disease prognosis, particularly in endometrial cancer. DR1 and DR2-like receptors are also expressed in a wide range of cell types, including cancer cells, immune and vasculature cells, suggesting that the dopaminergic system may be involved in tumour biology. Moreover, preclinical evidence reports dopamine receptor targeting as a novel anti-tumour strategy, with studies indicating that DRD1 agonists and DRD2 antagonists have antiproliferative and immune-activating effects in *in vitro* and *in vivo* cancer models (Section 1.5). The repurposing of clinically approved dopaminergic modulators offers a strategically advantageous route for drug discovery, bypassing extensive timelines and costs associated with *de novo* drug development. However, further investigations are required to understand the extent of the dopaminergic interactome within the tumour and TME of gynaecological cancers and to evaluate the efficacy of dopamine modulating drugs as an anti-cancer strategy.

Chapter Aims

This chapter aims to explore the dopaminergic landscape within gynaecological cancers and identify potent candidates for further *ex vivo* validation. The objectives are to:

- **Profile DR Expression:** Characterise the mRNA and expression patterns of D1-like and D2-like receptors in a panel of ovarian and endometrial cancer cell lines and xenografted tumour models.
- **Conduct High-Throughput Drug Screening:** Perform a targeted screen of DRD1 agonists and DRD2 antagonists to identify lead compounds that demonstrate significant anti-tumour efficacy.
- **Investigate the mechanism of DRD2 blocking:** Explore the effects of DRD2 blocking (Domperidone) on cell signalling pathways Raf/MEK/ERK and AKT/GSK3 and cancer cell motility, and in the presence of endocytosis inhibitors.

The findings from this chapter will establish the molecular foundation required to advance the most promising candidates into the OTSC system for more in depth TME investigations and patient-specific validation.

3.1 Methods

3.1.1 Cell Culture

The OVCAR8 cell line (CVCL_1629) and ISHIKAWA (CVCL_2529) cell lines were cultured as described earlier. The other gynaecological cancer cell lines: TOV112D (ATCC: CRL-11731) and HEC-1-A (ATCC: HTB-112) were grown in RPMI 4.5 g/mL glucose and SKOV3 (ATCC: HTB-77) and AN3CA (ATCC: HTB-111) cells were grown in DMEM 1 g/mL glucose/F12 (1:1). All media were supplemented with 10% foetal bovine serum (FBS, GIBCO), 2mM glutamine, 100 µg/mL Penicillin-streptomycin, and 1 µg/mL Amphotericin B (Life Technologies). Cells were cultured as per ATCC guidelines and kept at 37°C in a humidified environment of 5% CO₂.

3.1.2 Reverse Transcription qPCR

To confirm the expression of dopaminergic receptors in gynaecological cancer cell lines, reverse transcription quantitative polymerase chain reaction (RT-qPCR) assays were performed using TaqMan Gene Expression Assays. These assays consist of sequence-specific forward and reverse primers and a fluorogenic probe designed to detect target cDNA of dopaminergic receptors: DRD1 (Hs00265248_s1), DRD2 (Hs00241436_m1), DRD3 (Hs00364455_m1), DRD4 (Hs00609526_m1) and DRD5 (Hs00361234_s1).

Cell lines were grown in T-75 flasks to approximately 80% confluency, washed in PBS, trypsinised, and total RNA was extracted using the RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Scientific). For reverse transcription, 1 µg of total RNA was converted to complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). cDNA samples were either used immediately for qPCR analysis or stored at -20 °C until use.

For qPCR, the SensiFAST™ Probe Hi-ROX Kit (Bioline) was used with the 7900HT Fast Real Time PCR System (Applied Biosystems). During amplification, the gene-specific primers bind to the target cDNA sequence, and it is exponentially amplified by DNA polymerase with fluorescence measured in real time. Cycle threshold (Ct) is the number of

amplification cycles needed for the fluorescence to reach the threshold. Lower Ct values indicate higher initial target abundance, as samples containing greater amounts of target cDNA reach the fluorescence detection threshold in fewer amplification cycles during exponential PCR amplification. Gene expression was then normalised to the expression of the housekeeping gene GAPDH (Hs02786624_g1) and values calculated using the $2^{-\Delta Ct}$ method.

3.1.3 ELISA

To confirm the protein expression of DRD2 receptor in the cell lines, the protein was extracted and quantified using Enzyme-Linked Immunosorbent Assays (ELISA). ELISA is a sensitive and quantitative immunological technique used to detect and measure specific proteins in biological samples. The method is based on highly specific antigen–antibody interactions, in which the target protein (DRD2) is selectively recognised by antibodies immobilised on the well plate surface. Detection is achieved by adding an HRP-conjugated antibody that binds to the immobilised target protein and catalyses a blue-producing reaction upon addition of its substrate (tetramethylbenzidine; TMB). The reaction is then stopped using an acidic stop solution changing the colour from blue to yellow, and the intensity of the resulting colour is proportional to the concentration of the target protein and is quantified using a standard calibration curve.

For extraction, the cell lines were grown to approximately 80% confluency in T-75 flasks. The cells were then washed with PBS, trypsinised, and centrifuged at 300×g for 5 min. The supernatant was then removed and the pellet washed twice with PBS, centrifuged at 300×g for 5 min, and resuspended in 1mL PBS. The pellet then went through three cycles of rapid freeze-thawing in order to lyse the cell membranes. This gentle method of cell lysis was chosen in order to keep the protein in its native structure. After the final freeze-thaw, the sample was centrifuged at 13000×g for 5 min and the supernatant containing the protein was aliquoted into a clean Eppendorph tube and stored at -80°C until use.

The concentration of DRD2 protein was determined using a commercial ELISA kit (MyBiosource, Cat. No. MBS3800517), according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (TECAN), and DRD2

concentrations were calculated from the standard calibration curve prepared at 0, 75, 150, 300, 600, 1200 and 2400 pg/mL. The samples were then normalised by their total protein concentration as quantified by Bradford assay.

3.1.4 Bradford Assay

Protein concentration was determined using a colorimetric Bradford assay based on the absorbance shift of Coomassie Brilliant Blue G-250 (Bio-Rad). Under acidic conditions, the dye exists predominantly in a reddish-brown form and undergoes a shift to a blue form upon binding to proteins, primarily through interactions with basic and aromatic amino acid residues. The magnitude of the brown-to-blue colour change is proportional to the protein concentration present in the sample. To calculate protein concentration in test samples, known concentrations of BSA are assayed as calibrators at 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL BSA. The Bradford dye was made using 10 mL dye concentrate and 40 mL dH₂O and filtered using laboratory filter paper. Then, 5 µL sample (neat and at a 1:10 dilution) and BSA standards were pipetted onto a 96-well plate, 250 µL Bradford reagent were added per well and the plate gently tapped to mix. The plate was incubated at room temperature for 10 minutes, protected from light. Absorbance was then read at 595nm using a plate reader and the protein concentrations of test samples were calculated by interpolation from the BSA standard curve.

3.1.5 Protein Extraction for Western Blot

Cells were grown in 6-well plates (300,000 cells/well) until approximately 80% confluency. For later experiments with DRD2 antagonists, cells were grown with or without Domperidone for 24 and 48h. Cells were washed with PBS, lysed with RIPA buffer supplemented with activated sodium orthovanadate and a protease inhibitor (Sigma-Aldrich), and collected with a cell scraper. The collected cells were kept in an Eppendorph on ice for 15 min to ensure cell rupture. The cell extracts were then centrifuged 10 min at 13,000 RPM at 4°C. The supernatants were transferred to a fresh Eppendorph and stored in a -80°C freezer until use.

3.1.6 SDS-PAGE and Western blotting

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a technique in which proteins are separated according to their molecular weight. First, the concentrations of the lysates were calculated using the Bradford assay described above. Then, the lysate samples were diluted to 20 µg in dH₂O and 3x sample buffer containing SDS and samples were heated at 100°C for 5 min to denature them. Acrylamide gels (12% or 15%) were loaded onto the gel and subjected to an electrophoretic current of 100mV for 90 min at RT (BIO-RAD ; Mini-PROTEAN®).

The SDS-PAGE gel was then transferred to PVDF membranes by a wet transfer at 100mV for 70 min 4°C (ThermoScientific). Membranes were blocked for non-specific binding using Tris-buffered saline (TBS) supplemented with 0.2% Tween and 5% of BSA. Primary antibodies were incubated overnight rotating at 4°C at the appropriate concentration (see Table 16). The following day, membranes were washed in TBS and incubated with secondary antibodies, rotating for 1h at RT. The BIORAD Clarity Western Blot ECL substrate mix (peroxide and luminol enhancer) was then incubated for 1-5 min and imaged every second for around 30 seconds but sometimes up to 1 minute using ChemiDoc™ XRS+ with Image Lab™ software (BIO-RAD). Images were then analysed within the programme by quantifying the intensity of the band sizes. For semiquantitative measurements, the housekeeping gene GAPDH was stained on the same membranes, and the intensity of the target bands were used to quantify the protein levels relative to GAPDH band intensity.

Table 16. Antibodies used for Western Blot.

Antibody	Dilution	Commercial Source	Reference
DRD1	1:1000	Biotechne	MAB8276
DRD2	1:1000	Santa Cruz	sc-5303
DRD5	1:1000	MyBiosource	Mb176794
β -Arrestin-2	1:1000	Cell Signalling	3857
AKT	1:1000	Cell Signalling	9272
Phospho-AKT	1:1000	Cell Signalling	9271
p44/42 MAPK (Erk1/2)	1:1000	Cell Signalling	9102
Phospho-p44/42 MAPK (Erk1/2)	1:1000	Cell Signalling	4376
MEK 1/2	1:1000	Cell Signalling	9122
Phospho-MEK 1/2 (Ser217/221)	1:1000	Cell Signalling	9121
GSK3 beta	1:1000	Cell Signalling	9315
Phospho-GSK3 beta (Ser9)	1:1000	Cell Signalling	9323
GAPDH	1:500	Abcam	EPR16891
Goat Anti-Rabbit	1:10000	ThermoFisher	31460
Goat Anti-Mouse	1:10000	ThermoFisher	31430

3.1.7 Flow Cytometry for DRD1 and DRD2 Protein Expression

(a) Cell lines

To analyse DRD1 and DRD2 protein expression in ISHIKAWA and OVCAR8 cell lines, flow cytometry was employed. First, the cell lines were grown to approximately 80% confluency, washed, and trypsinised. The cells were then centrifuged at 300xg for 5 minutes and the pellet washed twice in 1mL 0.5% BSA/PBS with centrifugation at 300xg for 5 min. The pellet was then resuspended to $\sim 10^6/100 \mu\text{L}$ in 0.5% BSA/PBS and 100 μL of cell suspension was added to each test tube. The cells were then incubated with antibodies specific for DRD1 or DRD2. To ensure that antibody binding was target-specific, an isotype control tube was prepared using non-targeting antibodies of the same Ig family and conjugated fluorophore (see Table 17). Antibodies were incubated in the dark for 20 min at 4°C with constant agitation, followed by centrifugation. Then, the supernatant with excess antibody was removed, and the pellets washed and centrifuged

twice more in 1mL 0.5% BSA/PBS at 300xg for 5 min. Finally, each sample was resuspended with 500µL 0.5%BSA/PBS and incubated for 5 min with propidium iodide at a 1:10,000 final dilution to separate dead and alive cells. The samples were passed through a flow cytometer (Miltenyi) with lasers appropriately compensated for the fluorophores.

Table 17. Antibodies used for flow cytometry for DRD1 and DRD2

Antibody	Dilution	Commercial Source	Reference
D1DR (PE)	1:50	Biologend	366403
D2DR (AF-647)	1:50	AB online	ABIN6980343
DRD1 Isotype control: PE-IgG2b, k	1:50	Biologend	401207
DRD2 Isotype control: IgG-AF-647	1:50	AB online	ABIN1880987

(b) Whole Tumours

To analyse DRD1 and DRD2 protein expression in ISHIKAWA and OVCAR8 xenografted whole tumours, the tumours were excised and disaggregated using the Miltenyi Tumour Dissociation Kit according to the manufacturer's instructions and as described in Chapter 2 (2.1.10). The resulting pellet was then resuspended to $\sim 10^6/100 \mu\text{L}$ in 0.5% BSA/PBS and 100 µL of cell suspension was added to each test tube. The cells were then incubated with antibodies specific for DRD1 and DRD2 or their isotype control (see Table 17). Antibodies were incubated in the dark for 20 min at 4°C with constant agitation, centrifuged, the supernatant with excess antibody removed, and pellets washed and centrifuged twice more in 1mL 0.5% BSA/PBS at 300xg for 5 min. Finally, each sample was resuspended with 500µL 0.5%BSA/PBS and incubated for 5 min with propidium iodide at a 1/10,000 final dilution to separate dead and alive cells. The samples were passed through a flow cytometer (Miltenyi) with lasers appropriately compensated for the fluorophores.

3.1.8 High-Throughput Screening of Dopaminergic Receptor Modulators

To conduct a dopaminergic modulator drug screen, three DRD1/5 selective agonists (SKF83822, SKF82958, Fenoldopam), two DRD2 selective antagonists (Domperidone, L-741,626) and dopamine (native ligand; pan-DR agonist) were chosen (see Table 18).

ISHIKAWA and OVCAR8 cells were grown in 96-well plates in 100 μ L media (10^4 cells/well). All drugs were diluted and tested at 0, 1, 10, 25, 50, 75, 100, 200 μ M for 48h in the ISHIKAWA and OVCAR8 cell lines. To assess the cytotoxicity of the reagents, AlamarBlue assays were conducted by adding 10 μ L of AlamarBlue for 2h. The fluorescence (AlamarBlue) was read at 560/590 nm using the Infinite M200 Pro plate reader (TECAN). To calculate cell viability inhibition, the difference in readout between drug and control conditions were calculated and the IC₅₀ values were calculated from Dose-Response Inhibition curves in GraphPad.

Table 18. Concentration and mechanism of the DRD1 and DRD2 modulating drugs.

Drug Name	Mechanism	Stock Concentration	Manufacturer	Reference
SKF83822	DRD1/DRD5 Agonist	20mM in DMSO	Tocris	2075
SKF82958	DRD1/DRD5 Agonist	20mM in DMSO	Tocris	5719
Fenoldopam	DRD1/DRD5 Agonist	10mM in dH ₂ O	Sigma	SCL0198
Dopamine	DRD1-5 Agonist	10mM in dH ₂ O	Sigma	H8502
L-741,626	DRD2 Antagonist	20mM in DMSO	Tocris	1003
Domperidone	DRD2 Antagonist	20mM in DMSO	Cayman Chemical	18875

3.1.9 Mechanism of Domperidone: Endocytic Inhibitors

To determine if the efficacy of Domperidone is mediated through extracellular interactions rather than endocytic trafficking and the interaction with intracellular targets, internalisation pathways can be pharmacologically blocked during Domperidone administration. There are five primary endocytic pathways: clathrin-mediated endocytosis, caveolae-mediated endocytosis, macropinocytosis, phagocytosis and clathrin/caveolae-independent mechanisms. By selectively inhibiting these routes, it is possible to observe whether Domperidone-induced cell death is attenuated when the drug is restricted to the extracellular space.

First, OVCAR8 and ISHIKAWA cells were seeded at a density of 1×10^5 cell/mL (0.5 mL/well) in 24-well plates using the appropriate culture medium supplemented with 1% FBS, to ensure all cells were at the same point of their cell cycle. Following overnight incubation, the media was replaced with 10% FBS media containing specific endocytosis inhibitors at concentrations between 5-130 μ M (Table 19). These predetermined concentrations were chosen as they represented IC_{50} values found in literature and were tested to ensure they maintained cell viability after 48 h incubation (Tang et al. 2017). Following a 2 h pre-incubation with the inhibitors, Domperidone was added to the treatment groups to achieve a final concentration of 70 μ M, and the cells were incubated for 48 h. To establish a baseline for cellular response, experimental controls included wells containing inhibitor-only, media-only, and Domperidone-only treatments. Cells were incubated for 48 hours, after which cell viability was assessed by replacing the media with a 1X AlamarBlue solution for 2 hours. Fluorescence was measured at 560/590 nm using a plate reader. All conditions were conducted in duplicate and the experiment repeated in triplicate for each cell line.

Table 19. Concentration and pathway inhibition of the endocytosis inhibitor drugs

<i>Inhibitor</i>	<i>Pathway</i>	<i>Concentration in literature</i>	<i>Concentration selected</i>
<i>Chlorpromazine</i>	Clathrin-mediated endocytosis	3.5-35 µg/mL	5 µg/mL
<i>Nystatin</i>	Caveolae-mediated endocytosis	1.35-135 µM	50 µM
<i>Colchicine</i>	Pinocytosis	0.625-62.6 µM	300nM
<i>Amiloride</i>	Macro-pinocytosis	2.5-250 µM	65 µM
<i>Dynasore</i>	Clathrin-mediated endocytosis	15-80 µM	20 µM

3.1.10 Wound-Healing Migration Assay

To evaluate the functional impact of Domperidone on cellular motility, a wound-healing (scratch) assay was employed. The principle of this assay is to create a gap between two compartments of cells and assess the change in area of the gap in response to the pharmaceutical agent over time.

The assay was performed using 2-well culture inserts (81176, IBIDI), placed in 24-well plates. First, cells were seeded at a density of 5×10^4 cells per insert compartment in appropriate media supplemented with 10% FBS (Gibco) and incubated in standard culture conditions for 24h until 100% confluence was reached in both compartments. To inhibit cell proliferation, cells were pretreated with actinomycin D (A9415, Sigma-Aldrich) at a final concentration of 5 µg/mL for 2h. To prevent detached cells from settling within the wound healing area (gap between insert), culture media was carefully aspirated, and each compartment was washed with 50µL of 1X PBS. The culture insert was subsequently removed using sterilized tweezers to generate a defined cell-free gap. After removal of the insert, 300µL of the corresponding media supplemented with 1% FBS was added to each well. Cells were then treated with Domperidone at two different concentrations (10µM and 20µM). Duplicate wells were prepared for each experimental condition. Immediately after treatment addition, images were acquired using a light

microscope to document the baseline wound area (0h), which served as the control for subsequent quantification. Plates were then returned to the incubator, and wound closure was monitored over a total period of 48h by acquiring images at 0, 3, 6, 9, 12, 24, 31 and 48h after insert removal. The wound area was quantified using ImageJ Software with the Wound Healing Size Tool plugin. The central wound region was selected from each image, and the percentage of wound closure was calculated. Results are presented as mean values from three independent experiments.

3.1.11 Statistical Analysis

For the inhibitor assays, the effect of individual inhibitors on cell viability was first assessed using one-sample t-tests against a theoretical baseline of 100%, with p-values adjusted for multiple comparisons using the Bonferroni correction. To analyse the impact of inhibitor treatments within the Domperidone-treated groups, linear mixed-effects models (LMM) were employed. In these models, the inhibitor treatment was treated as a fixed effect, while the Experiment ID was included as a random effect to account for inter-replicate and inter-plate variability. Significant effects within the LMM were further explored using Dunnett's post-hoc tests to compare each inhibitor treatment back to the Domperidone-only reference group. For all tests, a p-value < 0.05 was considered statistically significant. Statistical analyses were performed using R (version 4.1.2) with the lme4, car, and lmerTest packages.

To analyse western blot protein expression data, the dataset was partitioned by individual protein targets, and linear models were fitted to evaluate the effect of the experimental group on normalised expression levels. A Type II Analysis of Variance (ANOVA) was used to determine the significance of the main effects, followed by post-hoc comparisons of each experimental group against the designated control using estimated marginal means with Dunnett-style adjustments. Prior to testing, data were inspected for normality using Shapiro-Wilk tests and homogeneity of variance of the model's residuals. For differences in receptor expression levels between the cell line OTSC models, unpaired T-tests were employed.

3.2 Results

3.2.1 DRD2 is the Predominant Dopamine Receptor Across HGSOC and EC Models

To characterise dopamine receptor expression across gynaecological cancer models, mRNA expression of DRD1-5 was quantified by RT-qPCR in six cell lines representing ovarian (OVCAR8, SKOV3, TOV112D) and endometrial (AN3CA, HEC1a, ISHIKAWA) cancer subtypes. Both OVCAR8 and ISHIKAWA cell lines exhibited relatively low DRD1 expression compared to the other gynaecological cancer cell lines (Figure 21A). Between these two cell lines, OVCAR8 presented a 3.29-fold increase in DRD1 expression compared to ISHIKAWA cells. Conversely, out of all the gynaecological cell lines tested in this study, OVCAR8 and ISHIKAWA also had relatively the highest expression of DRD2, exhibiting a huge 8.2-fold and 10.4-fold increase in expression, respectively, compared to the lowest expressing line TOV112D. Between the two primary models, OVCAR8 DRD2 expression was approximately 22% lower than ISHIKAWA (Figure 21B). Interestingly, OVCAR8 had more DRD1 expression than DRD2 (6.77×10^{-5} vs. 1.86×10^{-5}), whereas ISHIKAWA had almost an even expression of DRD1 and DRD2 (2.06×10^{-5} vs. 2.37×10^{-5}).

To confirm findings, DRD1 and DRD2 protein expression was assessed by Western blot, flow cytometry and ELISA in OVCAR8 and ISHIKAWA cell lines (Figure 21D, F-H). In contrast to transcriptomic results, flow cytometry analysis demonstrated that OVCAR8 cultures had approximately a 32% increase (5.41% vs. 4.08) in DRD2+ cells compared to ISHIKAWA cultures (Figure 21G). Whereas, as seen in the flow cytometry plots, in both cell lines there were no DRD1+ cells detected (Figure 21H). This trend was supported in western blot images, where the 51 kDa band corresponding to DRD2 showed a greater intensity in OVCAR8 samples compared with ISHIKAWA, further indicating higher expression levels (Figure 21D). Consistent with mRNA and flow cytometry results, DRD1 bands were comparably faint between cultures, indicating low expression. Interestingly, the DRD2 ELISA provided an inverse result, quantitatively demonstrating that ISHIKAWA had 69% higher levels of DRD2 relative to OVCAR8 (Figure 21F). While these discrepancies may have originated from differences in methodology e.g., total cellular levels vs. surface expression, the data collectively confirms DRD2 is the dominant receptor in both models with minimal DRD1 protein expression.

3.2.2 DRD1 Expression Prevalence Increases in the OVCAR8 OTSC Model

To compare DRD1 and DRD2 protein expression levels in the OVCAR8 and ISHIKAWA xenografted whole tumours or tumour slice models, tumours were resected, sliced (if applicable), disassociated and analysed with RT-qPCR, flow cytometry or Western Blot. In contrast to cell line results, OVCAR8 whole tumours demonstrated an extremely high expression level of DRD1 with a mean value of 1.97×10^{-3} (AU relative to GAPDH) compared with the original OVCAR8 cell line (6.77×10^{-5}), representing a massive 29-fold increase (Figure 21C). Paradoxically, in ISHIKAWA slices DRD1 mRNA levels were undetectable, consistent with transcriptomic, Western blot and flow cytometry analyses in the original cell line (Figure 21). However, DRD2 levels appeared somewhat comparable between OVCAR8 tumour slices (5.6×10^{-6}) and the original cell line (1.86×10^{-5}), with a modest 3-fold decrease. Moreover, between OVCAR8 and ISHIKAWA tumour slices DRD2 levels were also comparable (5.8×10^{-6} vs. 5.21×10^{-6}) (Figure 21C). Interestingly, both mRNA and Western blot analysis presented a similar observation, with a greater DRD1 intensity compared to DRD2 in the OVCAR8 tumour slices (Figure 21C, E).

Consistent with the earlier observations, flow cytometry analysis also revealed distinct receptor expression levels between xenografts. Similar to RT-qPCR results, OVCAR8 xenografted tumours demonstrated higher DRD1 levels than ISHIKAWA, marked by the small presence of 0.2% DRD1-positive cells, whereas this population was absent in ISHIKAWA tumours (Figure 21 I-J). However, ISHIKAWA tumours were found to have ~50% more DRD2+ cells than OVCAR8 (2.7% vs. 1.8%), contradicting mRNA analysis. From the DRD2+ population identified in OVCAR8 and ISHIKAWA tumours, these cells were characterised by population-specific markers, such as epithelial cells (EpCAM+), immune cells (CD45+) and endothelial cells (CD31+). Interestingly, the majority of OVCAR8 DRD2+ cells belonged to the immune cell fraction (43.5%), followed by epithelial cells (29.6%), and a small percentage of endothelial cells (0.5%). There was also a high number (26.4%) of OVCAR8 DRD2+ that belonged to an uncategorised tumour population (EpCAM-/CD45-/CD31-). In contrast, DRD2+ cells within ISHIKAWA tumours consisted predominately of epithelial cells (88.9%) with only a minor fraction belonging to immune (CD45+, 2.6%) and endothelial (CD31+, 0.51%) cells (Figure 21J).

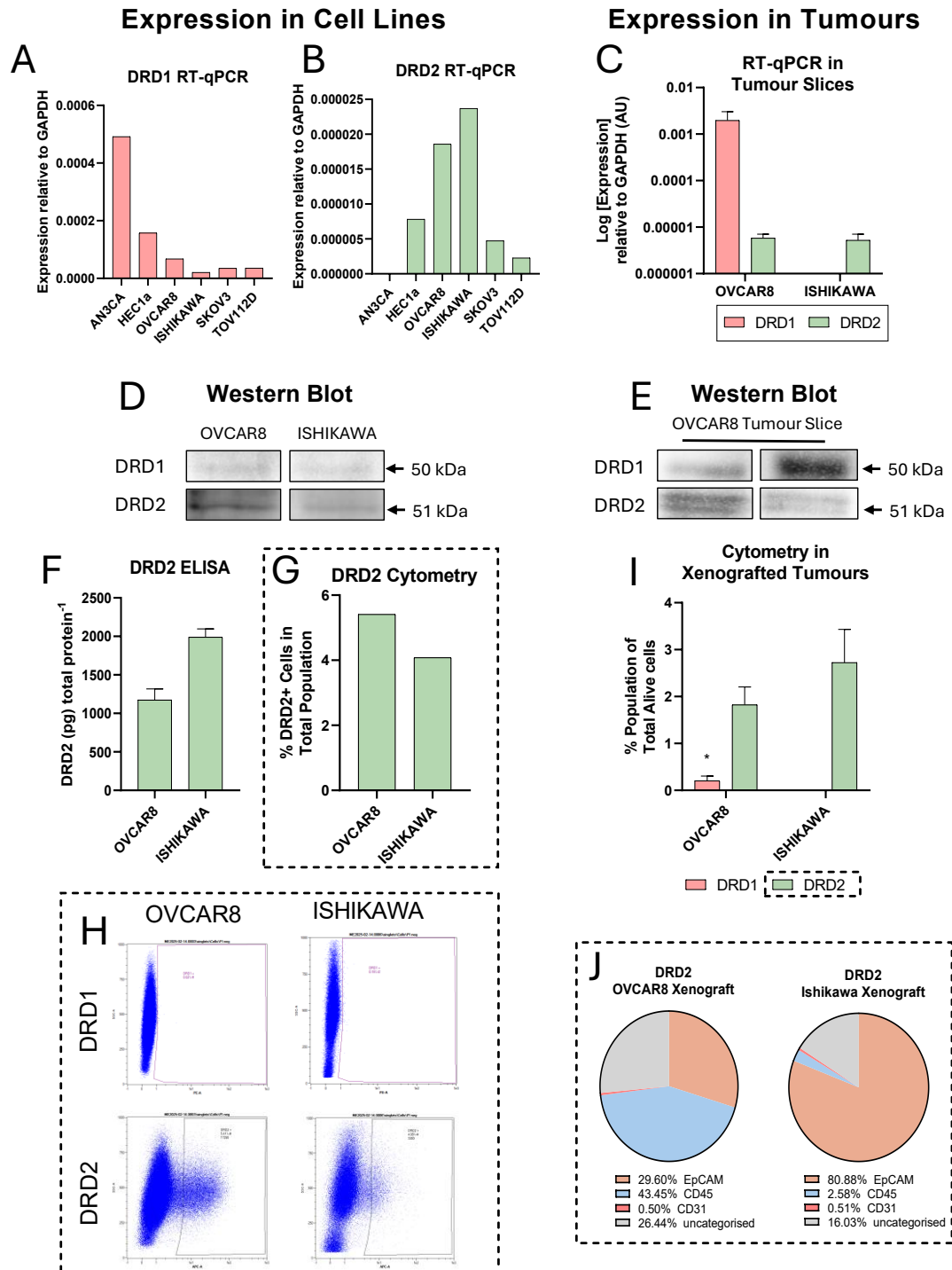


Figure 21. (A) DRD1 or (B) DRD2 mRNA quantification in gynaecological cancer cell lines or (C) OVCAR8 and ISHIKAWA tumour slices (DRD1; pink and DRD2; green). (D) Western blots of DRD1 and DRD2 in protein extracts of OVCAR8 and ISHIKAWA cell lines or (E) OVCAR8 tumour slices ($n=2$). (F) DRD2 protein expression in OVCAR8 and ISHIKAWA cell lines measured by ELISA, $n=2-4$ or (G) Flow cytometry. (H) Representative flow cytometry plots of Fig. G and gating strategy for DRD1+ and DRD2+ cells. (I) Flow cytometry quantification of DRD1+ (pink) and DRD2+ (green) cells in OVCAR8 and ISHIKAWA tumours (* $p<0.05$ using an unpaired T-test). (J) Categorisation of the DRD2+ population from Fig. I (dotted box) into their corresponding populations (epithelial, immune, endothelial or uncategorised). Graphs plotted using mean $\pm$ SEM.

3.2.3 DRD2 Blockades Identified as a Promising Anti-Tumour Strategy

To evaluate the functional significance of DRD1 and DRD2 expression, a pharmacological screen was conducted using three DRD1/5 selective agonists (SKF83822, SKF82958, Fenoldopam), two DR2-like selective antagonists (Domperidone, L-741,626) and the pan-DR agonist dopamine were tested in the OVCAR8 (Figure 22) and ISHIKAWA (Figure 23) cell lines.

Treatment with dopamine and the selective agonist Fenoldopam failed to reduce cell viability at any tested concentration (up to 200 μ M). In contrast, both DRD1/5 agonists SKF83822 and SKF82958 reduced cell viability in the cell lines, with comparable IC_{50} values of 100.0 and 95.4 μ M in OVCAR8 (Figure 22), and 110.9 and 107.7 μ M in ISHIKAWA (Figure 23) respectively (Table 20).

In contrast, the DR2-like antagonists exhibited significantly higher potency. Domperidone and L-741,626 displayed effective inhibition in the cell lines at lower concentrations than the DRD1 agonists. In OVCAR8 cells, Domperidone reached 50% viability at a calculated IC_{50} values of 72 μ M, whereas L-741,626 reached this point at a lower concentration of 59.7 μ M (Figure 22). Concentrations were almost identical for ISHIKAWA cells, with marginally lower IC_{50} values of 71.1 μ M and 55.9 μ M, respectively (Figure 23).

Despite the relative potency of L-741,626, its clinical utility is limited as it is not currently FDA or EMA-approved for patient use. Furthermore, this agent displayed a remarkably steep dose-response curve; for example, in OVCAR8 cells, viability dropped dramatically from 115.8% at 50 μ M to just 5.1% at 75 μ M. This narrow therapeutic window suggests an ‘on-off’ cytotoxic threshold rather than a gradual dose-dependent inhibition, which is concerning when calculating appropriate dosages for *in vivo* models or patients. Consequently, Domperidone was selected as a promising agent for further testing within our 2D and 3D models.

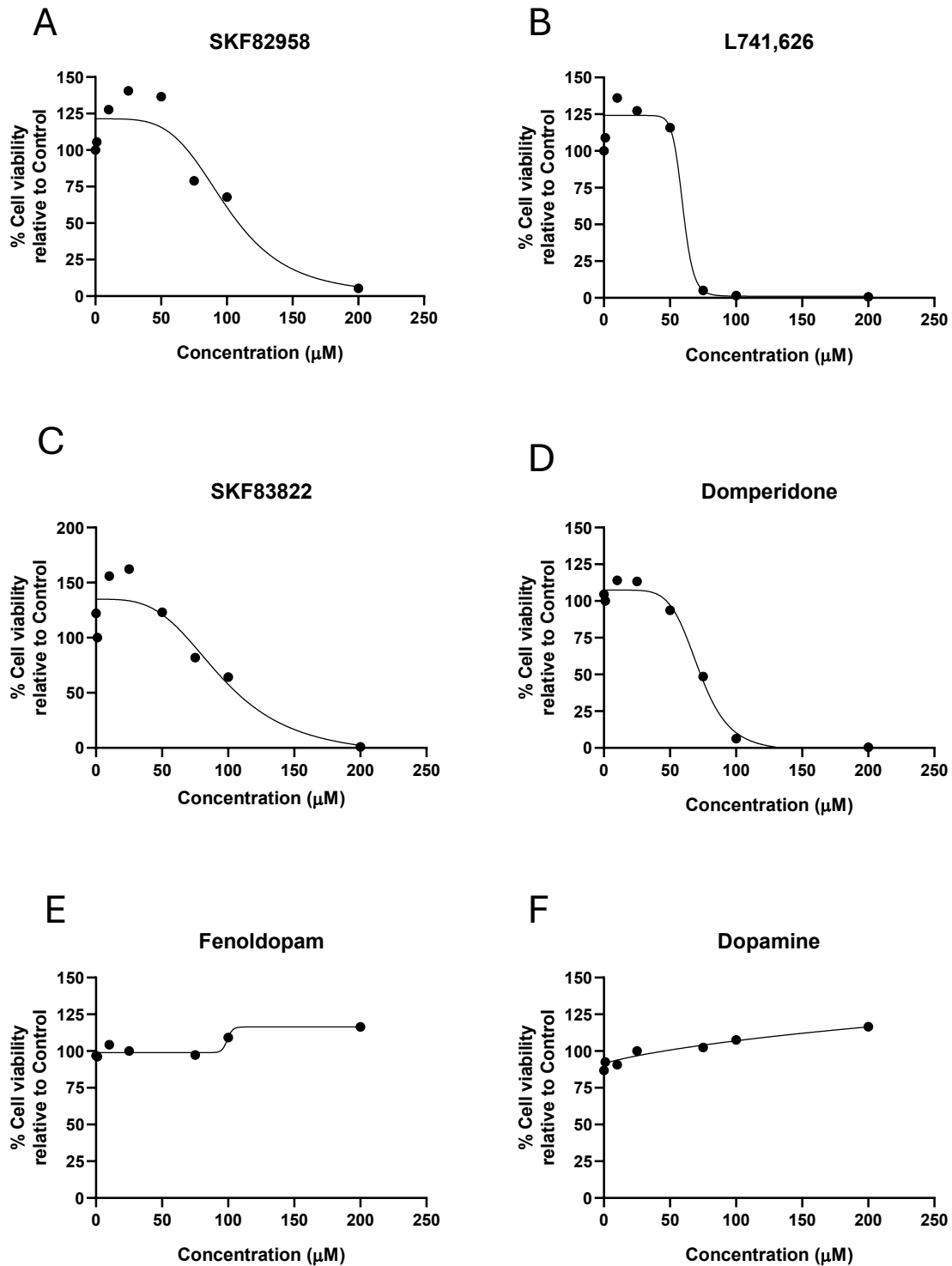


Figure 22. IC₅₀ of Dopaminergic Drugs in OVCAR8 cell line. The inhibitory effects of (A) SKF83822, (B) L-741,626, (C) SKF82958, (D) Domperidone, (E) Fenoldopam and (F) Dopamine after 48h incubation of drug at concentrations 0-200μM in OVCAR8 cells seeded at 1×10^5 cells/well in 96-well plates measured by AlamarBlue.

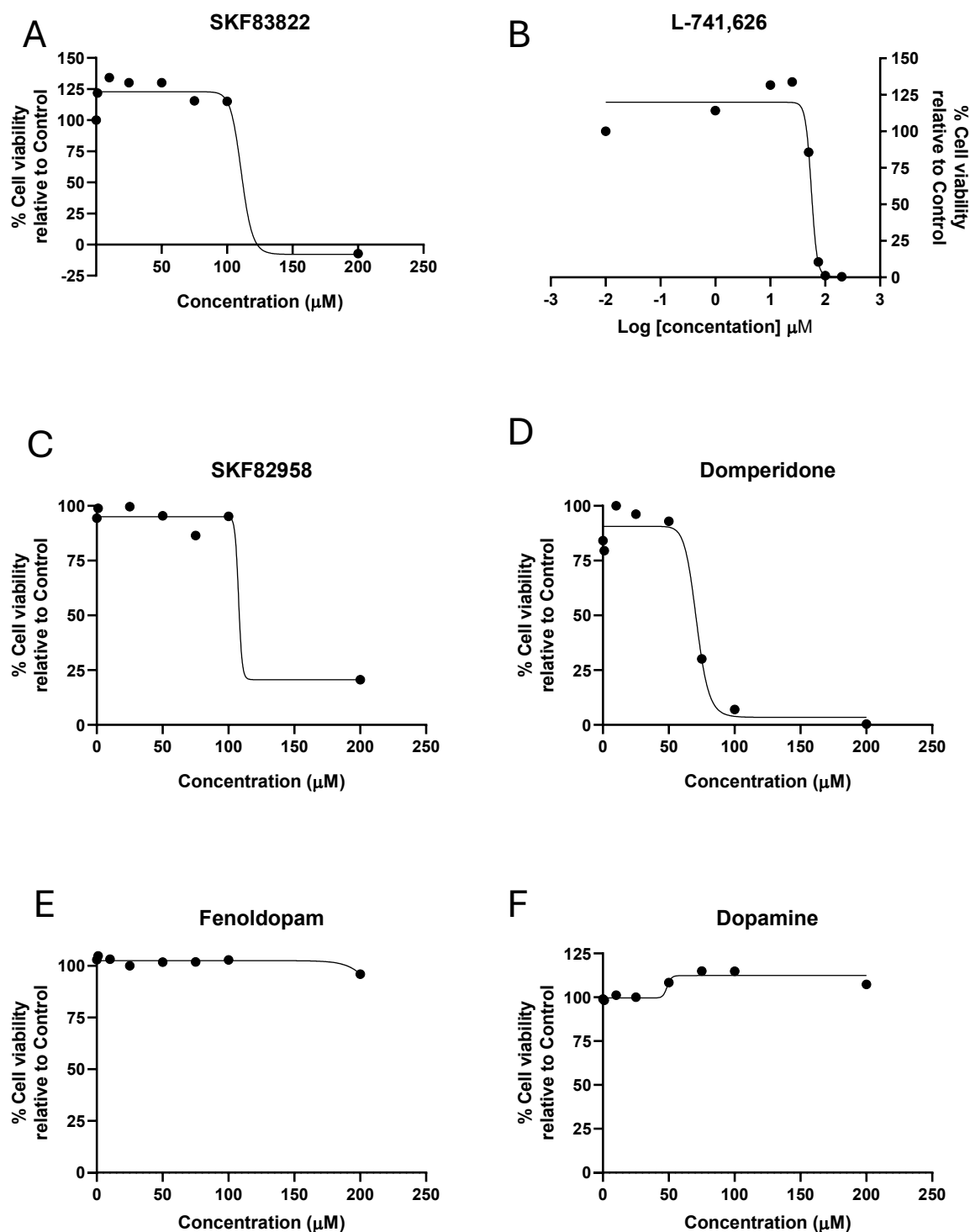


Figure 23. IC₅₀ of Dopaminergic Drugs in ISHIKAWA cell line. The inhibitory effects of **(A)** SKF83822, **(B)** L-741,626, **(C)** SKF82958, **(D)** Domperidone, **(E)** Fenoldopam and **(F)** Dopamine after 48h incubation of drug at concentrations 0-200μM in ISHIKAWA cells seeded at 1×10^5 cells/well in 96-well plates measured by AlamarBlue.

Table 20. Summary of the IC₅₀ at 48h of Dopaminergic Drugs in OVCAR8 and ISHIKAWA cell lines, their mechanism and their FDA and EMA approval status.

Drug Name	Mechanism	FDA/ EMA Approved	IC ₅₀ (μM)	IC ₅₀ (μM)
			OVCAR8	ISHIKAWA
SKF83822	DRD1/DRD5 Agonist	No	99.96	110.90
SKF82958	DRD1/DRD5 Agonist	No	95.40	107.70
Fenoldopam	DRD1/DRD5 Agonist	FDA Approved	>200	>200
Dopamine	DRD1-5 Agonist	n/a	>200	>200
L-741,626	DRD2-4 Antagonist	No	59.65	55.87
Domperidone	DRD2-4 Antagonist	EMA Approved	71.98	71.14

3.2.4 Domperidone Efficacy not Affected by Endocytosis Blockades

To ascertain whether Domperidone is externally driving cellular cytotoxicity, or if it is being endocytosed and trafficked to an internal target, we tested Domperidone at 70 μM alongside a range of endocytic inhibitors (20 μM Dynasore, 8 μM Chlorpromazine, 50 μM Nystatin, 65 μM Amiloride, or 300nM Colchicine).

OVCAR8 cells treated with inhibitors Dynasore, Chlorpromazine, Nystatin, and Amiloride had comparable viability values of 72-96% compared to the media-only control wells set to a baseline of 100% ($p > 0.05$). Whereas Colchicine was the only inhibitor to significantly reduce cellular signal (adjusted p -value = 0.0143) (Figure 24A). This indicated that Colchicine is cytotoxic to the cells, even at this relatively low concentration. When OVCAR8 cells were treated with Domperidone alone, cell viability reached a mean of 6.9%. This condition was used to compare wells treated with both inhibitor and

Domperidone at 70 μ M to ascertain whether the addition of the inhibitor prevented Domperidone-induced cell death. However, cells cotreated with both Domperidone and inhibitors displayed comparable viabilities to the Domperidone-only group ranging between 5.6-7.6% ($p>0.05$), except for the Domperidone/Nystatin group which had a slight but non-significant increase in viability to a value of 9.7% ($p=0.622$) compared to the Domperidone-only control (6.9%) (Figure 24A). When normalising Domperidone/inhibitor cotreated cells to the inhibitor-only wells, linear mixed-effects modelling showed that most of the inhibitors did not prevent Domperidone-induced death ($p>0.05$) with differences ranging from -0.204 to +3.4, except colchicine/Domperidone co-treated cells which had approximately 3.3-fold higher viability than Domperidone-only treated cells ($p=0.0089$) (Figure 24C).

Similar to OVCAR8 cell experiments, ISHIKAWA cells treated with inhibitors Dynasore, Chlorpromazine, Nystatin, and Amiloride also displayed comparable viabilities to media-only control with values of 81.0-111.4% ($p>0.05$), whereas again colchicine at 300nM caused a significant decrease in viability with a mean of 22.4% (adjusted p -value = 0.0145) (Figure 24B). When ISHIKAWA cells were treated with Domperidone alone, cell viability reached a mean of 29.0% and all inhibitor/Domperidone conditions achieved a comparable or lower viability (8.9-20.0%). Of note, Dynasore/Domperidone treated cells experienced a significant 3.2-fold loss compared with Domperidone alone ($p=0.0014$). Similarly, Amiloride/Domperidone treated cells had an almost 6-fold loss of viability compared with Domperidone alone, however this result was just shy of the statistical threshold ($p=0.0532$) (Figure 24B). When normalising Domperidone/inhibitor cotreated cells to the inhibitor-only wells, linear mixed-effects modelling showed that all the inhibitors did not prevent Domperidone-induced death ($p>0.05$) with many of the mean viabilities being from 5.11 to 12.65 units lower than Domperidone-only condition (Figure 24D). However, like with OVCAR8 cells, ISHIKAWA cells co-treated with colchicine and Domperidone had approximately 2-fold higher viability than Domperidone-only treated cells ($p=0.0320$).

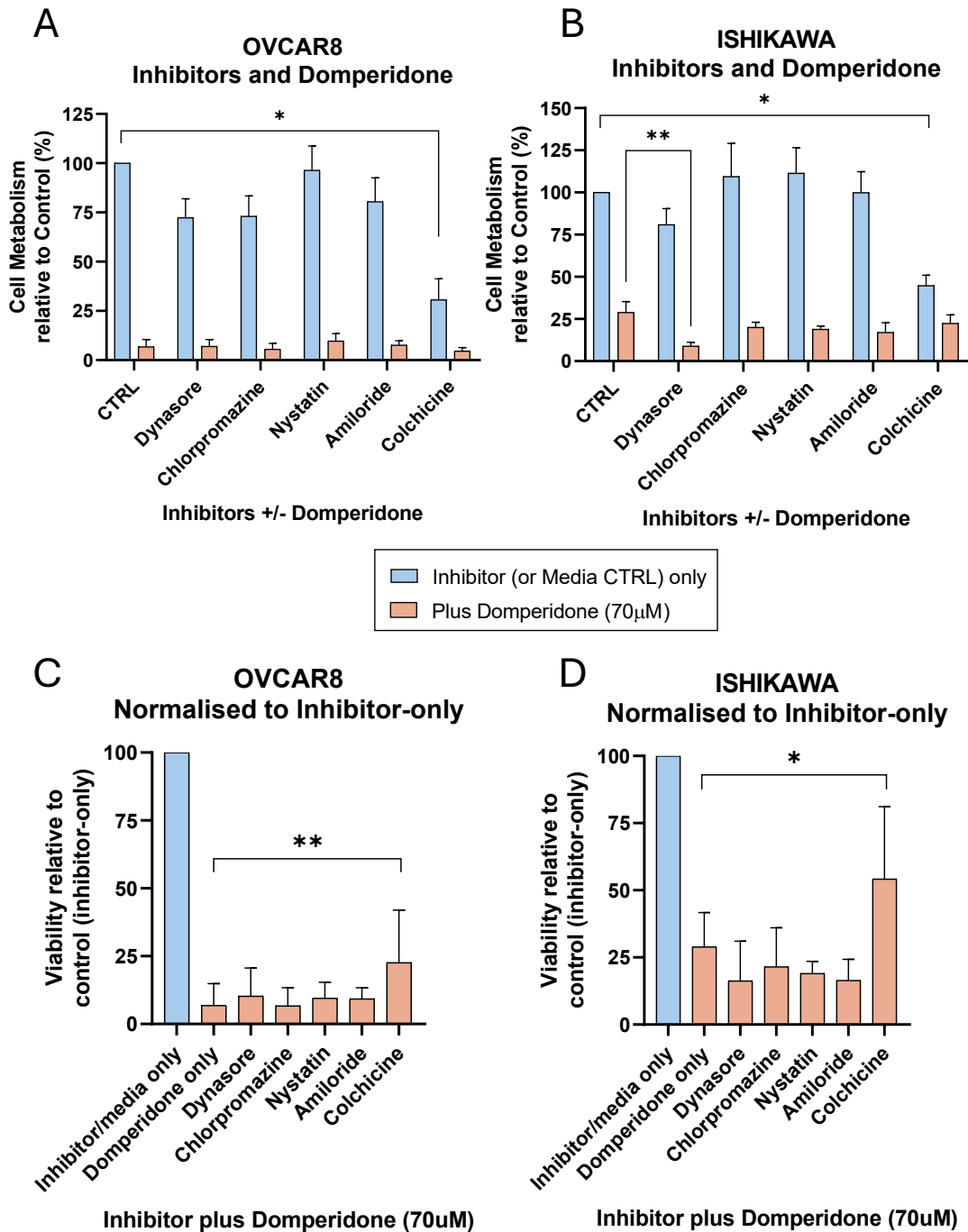


Figure 24. Endocytic inhibition on Domperidone-induced cytotoxicity in OVCAR8 and ISHIKAWA cells. (A) Viability of OVCAR8 and (B) ISHIKAWA cells pre-treated with endocytic inhibitors (20 μM Dynasore, 8 μM Chlorpromazine, 50 μM Nystatin, 65 μM Amiloride, or 300 nM Colchicine) with or without 70 μM Domperidone relative to media-only control (100%). Significance was determined via one-sample t-tests against a theoretical mean of 100% with Bonferroni correction for multiple comparisons ($n=4-5$, $*p<0.05$). (C) Viability of OVCAR8 cells and (D) ISHIKAWA cells co-treated with inhibitor and 70 μM Domperidone relative to respective inhibitor-only controls. Significance determined using a Linear Mixed Model and Dunnett's post-hoc test for comparisons against the Domperidone-only control ($n=4-5$, $*p<0.05$).

3.2.5 Targeted DRD2 Inhibition Significantly Impairs Endometrial Cancer Motility

An *in vitro* wound-healing assay was performed in OVCAR8 and ISHIKAWA cell lines to evaluate effects on wound healing after Domperidone treatment. Four treatment conditions were established: untreated control (media-only), actinomycin D alone (proliferation inhibitor), and actinomycin D combined with either Domperidone at 10 μ M or 20 μ M. Cells were grown in dual-compartment inserts separated by a gap which functions as the wound healing space. The change in wound area in response to the different treatments was calculated using semi-automated image quantification and presented as a percentage relative to the starting area (Figure 25).

In OVCAR8 cells, all groups showed progressive wound closure over time, with comparable closure areas at both early and late time points. In the untreated control, the wound area declined from 100% at 0h to 75.8% at 12h, 40.1% at 24h, and 9.6% at 48h, implying approximately 90% of closure after the 48h monitoring period (Figure 25A). Treatment with actinomycin D alone produced a similar profile with only minor non-significant increases in wound area at each time point after 3h, with a 48h wound closure area of 14.3%. Adding Domperidone had minimal impact on migration. Although both treatment groups displayed some decline in wound closure, none of these differences reached statistical significance thresholds in comparison to the Actinomycin Control ($p > 0.05$). For instance, at 6h the 10 μ M and 20 μ M Domperidone group maintained an area of 95.4% and 96.1%, respectively, compared to the Actinomycin group's value of 91.5%. Similarly, at 12h values were also marginally higher with an area of 85% and 86.7% compared to the Actinomycin Control (77.9%). Whereas at 48h closure responses varied with the 10 μ M group maintaining less area of 7.3% and 20 μ M with a greater wound area of 18.7% compared to the Actinomycin control of 14.3% (Figure 25A). Moreover, qualitatively, wound images showed similar closures of the gap in all groups at each time point, with no obvious change in cell migration pattern due to Domperidone effect (Figure 25B).

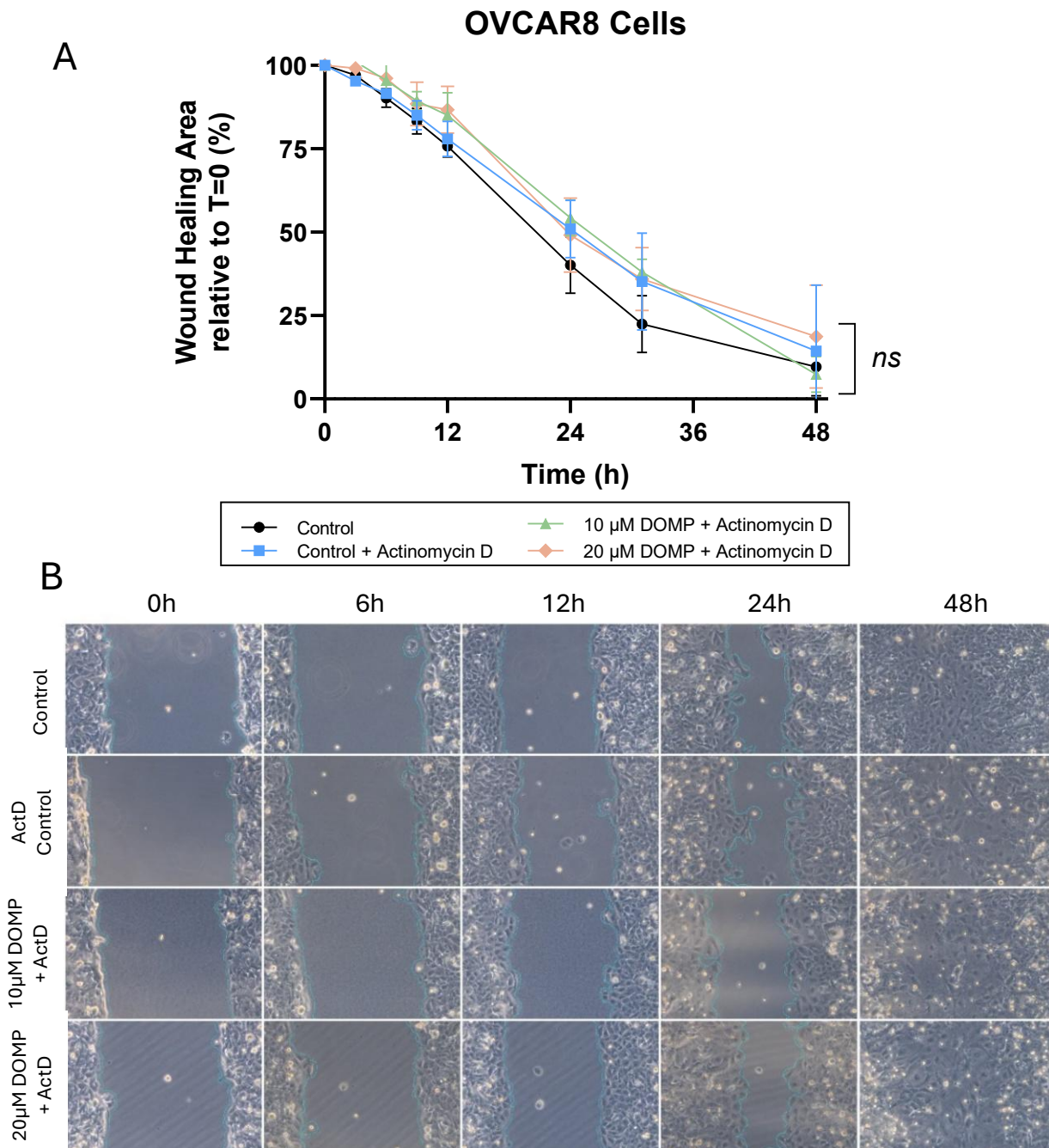


Figure 25. Effect of Domperidone on wound healing in OVCAR8 cell line. (A) Quantitative analysis of wound healing in OVCAR8 cells treated with media only (Control), Actinomycin D (ActD; proliferation inhibitor) alone or in combination with 10 μ M or 20 μ M Domperidone. Wound area is expressed as a percentage relative to the initial wound area at T=0. Data points represent the mean $\pm$ SEM of three independent experiments. Statistical analysis was performed using linear mixed modelling with post-hoc comparisons against Actinomycin Control Group; ns = not significant. **(B)** Representative phase-contrast micrographs showing the progression of wound closure at 0, 6, 12, 24, and 48 hours post-insert removal for each treatment condition.

In contrast, ISHIKAWA cells demonstrated distinct changes in cell migration upon treatment with Domperidone (Figure 26). In the untreated control group, the remaining wound fell from 100% at 0h to only 1.1% at 48h, indicating the wound had almost completely closed. Actinomycin D Control alone demonstrated minor non-significant changes compared to the media-only Control, with the final area at 48h estimated to be approximately 8.8% of the starting area. In contrast, ISHIKAWA cells treated with 10 μ M Domperidone/actinomycin D exhibited significantly less closure at 24h ($p < 0.0001$), 31h ($p < 0.0001$), and 48h ($p < 0.0001$) with wound areas estimated at 65.8%, 58.3% and 42.7%, compared with the Actinomycin-only Control values of 46.2%, 34.7%, and 8.8%. Similarly, ISHIKAWA cells treated with 20 μ M Domperidone and actinomycin D also exhibited significantly less closure at 24h ($p < 0.0007$), 31h ($p < 0.0001$), and 48h ($p < 0.0001$) with wound area ranging between 62.5% at 24h to 48.7% at 48h. However, there were no significant differences between the two Domperidone treatment groups ($p > 0.05$) (Figure 26A).

Comparing the two cell lines, OVCAR8 cells achieved nearly complete wound closure under all conditions by 48h, including with addition of Domperidone. Whereas in ISHIKAWA cells only the control wounds were almost completely closed, while Domperidone-treated groups were still partially open, as seen in Figure 26B. Statistical testing revealed that ISHIKAWA wounds were significantly bigger than OVCAR8 wound areas with Domperidone treatment at 24h for 20 μ M ($p = 0.0319$), and at both treatment concentrations at 31h (10 μ M: $p = 0.002$, 20 μ M $p = 0.0004$) and 48h (10 μ M: $p < 0.0001$, 20 μ M: $p < 0.0001$), demonstrating Domperidone was more effective at inhibiting migration in the endometrial cancer cell line compared to the ovarian cancer cell line.

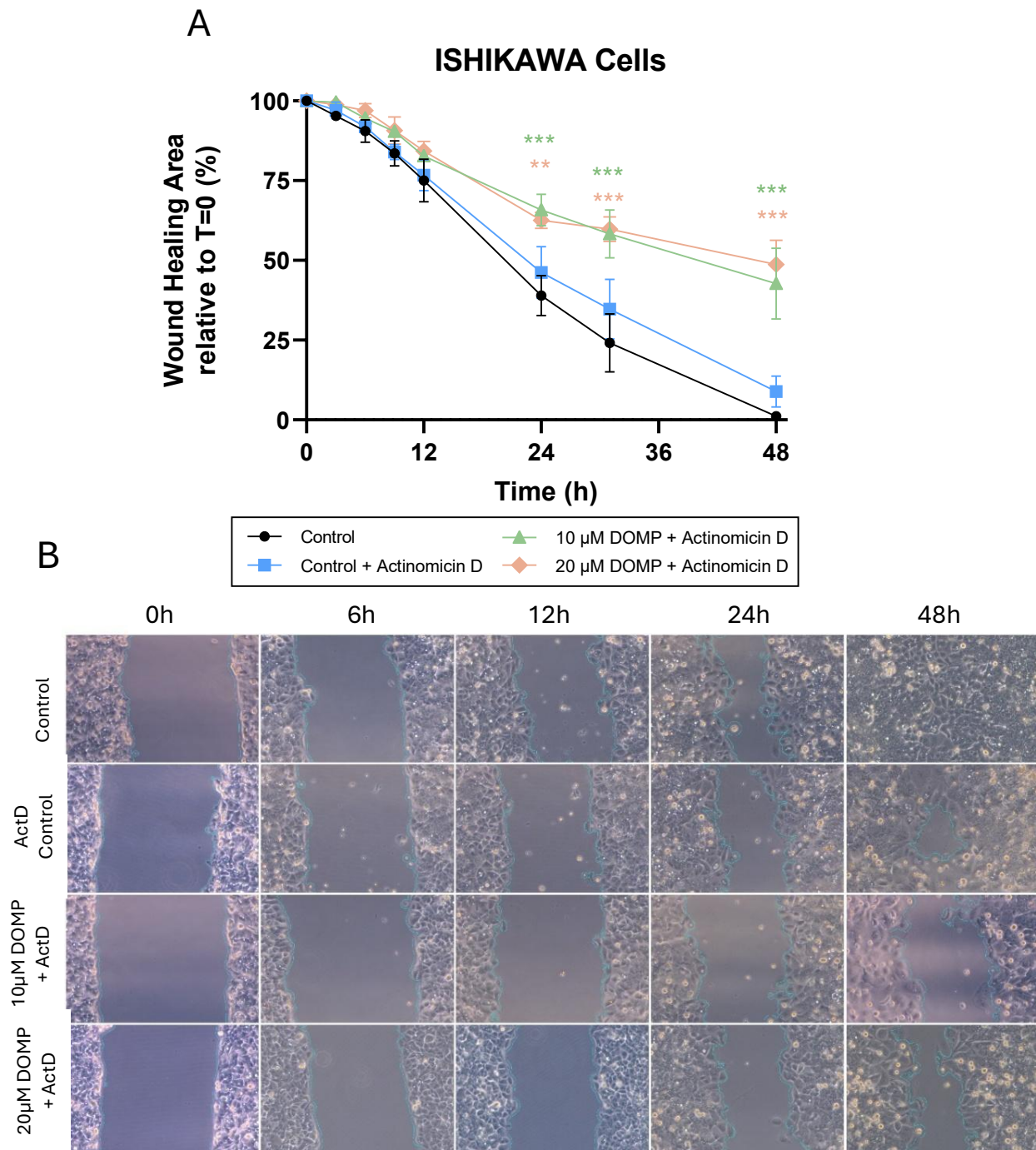


Figure 26. Effect of Domperidone on wound healing in ISHIKAWA cell line. (A) Quantitative analysis of wound healing in ISHIKAWA cells treated with media only (Control), Actinomycin D (proliferation inhibitor) alone or in combination with 10μM or 20μM Domperidone. Wound area is expressed as a percentage relative to the initial wound area at T=0. Represented as mean $\pm$ SEM of three independent experiments. Statistical analysis was performed using linear mixed modelling with post-hoc comparisons against Actinomycin Control Group; ** $p < 0.01$, $p < 0.0001$. **(B)** Representative phase-contrast micrographs showing the progression of wound closure at 0, 6, 12, 24, and 48 hours post-insert removal for each treatment condition.

3.2.6 OVCAR8 and ISHIKAWA Cells Exhibit Opposing Signalling Responses to DRD2 Antagonism.

To assess the downstream mechanisms of DRD2 blocking within the ovarian (OVCAR8) and endometrial (ISHIKAWA) cancer cells, the cell lines were treated with Domperidone at 35 μ M Domperidone for 24h. This incubation time and concentration was selected in order to maintain partial cell viability, as higher parameters led to insufficient protein levels for quantification. Changes in protein expression of dopaminergic receptors and intracellular molecules involved in MEK/ERK (MAPK) and AKT/GSK-3 signalling pathways were immunoblotted and quantified (Figure 27).

Analysis of Western blots revealed distinct cell line-dependent shifts in dopaminergic receptor levels and their downstream effector signalling molecules. OVCAR8 cells experienced decreases in both DRD1 (-36.7%) and DRD2 (-22.0%) protein levels and approximately a 19% rise in DRD5 following 24h treatment with 35 μ M Domperidone, compared to the media-only control (Figure 27A). In contrast, ISHIKAWA cells demonstrated an opposing response with a 20% increase in DRD2 and a 23% decrease in DRD5, alongside a smaller DRD1 (-23.6%) protein downregulation (Figure 27B). Protein levels of the DRD2 scaffold protein β -arrestin-2 were also distinctly altered; while OVCAR8 cells demonstrated an 8.7% rise in expression, ISHIKAWA cells had a 34.2% decrease upon treatment (Figure 27).

When exploring effector molecules involved in the Raf/MEK/ERK (MAPK) pathway, some signs of downregulation in this signalling cascade was observed. OVCAR8 cells treated with 35 μ M Domperidone demonstrated marked decreases in both total MEK levels (-29.3%) and phosphorylated-MEK levels (-63.9%) compared to the Control condition (Figure 27A). This was similar for ISHIKAWA cells which also had a downregulation of MEK (-31.1%) and p-MEK (-20.2%) (Figure 27B). However, in OVCAR8 cells, while total ERK levels decreased by -18.7%, p-ERK in its activated phosphorylated form increased marginally by 7.6% in the 35 μ M Domperidone group. In contrast, ISHIKAWA cells had an opposing response to OVCAR8 cells when looking at ERK and p-ERK levels, with values of +20.8% and -58.7%, respectively.

Signalling molecules within the PI3K/AKT/GSK-3 signalling pathway exhibited some signs of downregulation in OVCAR8 cells but not in ISHIKAWA. In OVCAR8 cells treated with Domperidone total AKT increased by 38.4%, whereas p-AKT in its active phosphorylated state diminished by 8.6%, compared to the control. Moreover, total GSK-3 β and phosphorylated (Ser9) GSK-3 β in its inhibited state were increased (+51.5% and +82.3, respectively) (Figure 27A). Again, ISHIKAWA cells exhibited a completely contradictory response, with a rise in p-AKT (+9.2%), and reductions in total AKT (-28.3%), total GSK-3 β (20.3%) and inactive p-GSK-3 β (-5.6%) (Figure 27B). This indicates both cell lines respond in distinct and opposing manners to DRD2 antagonism therapy.

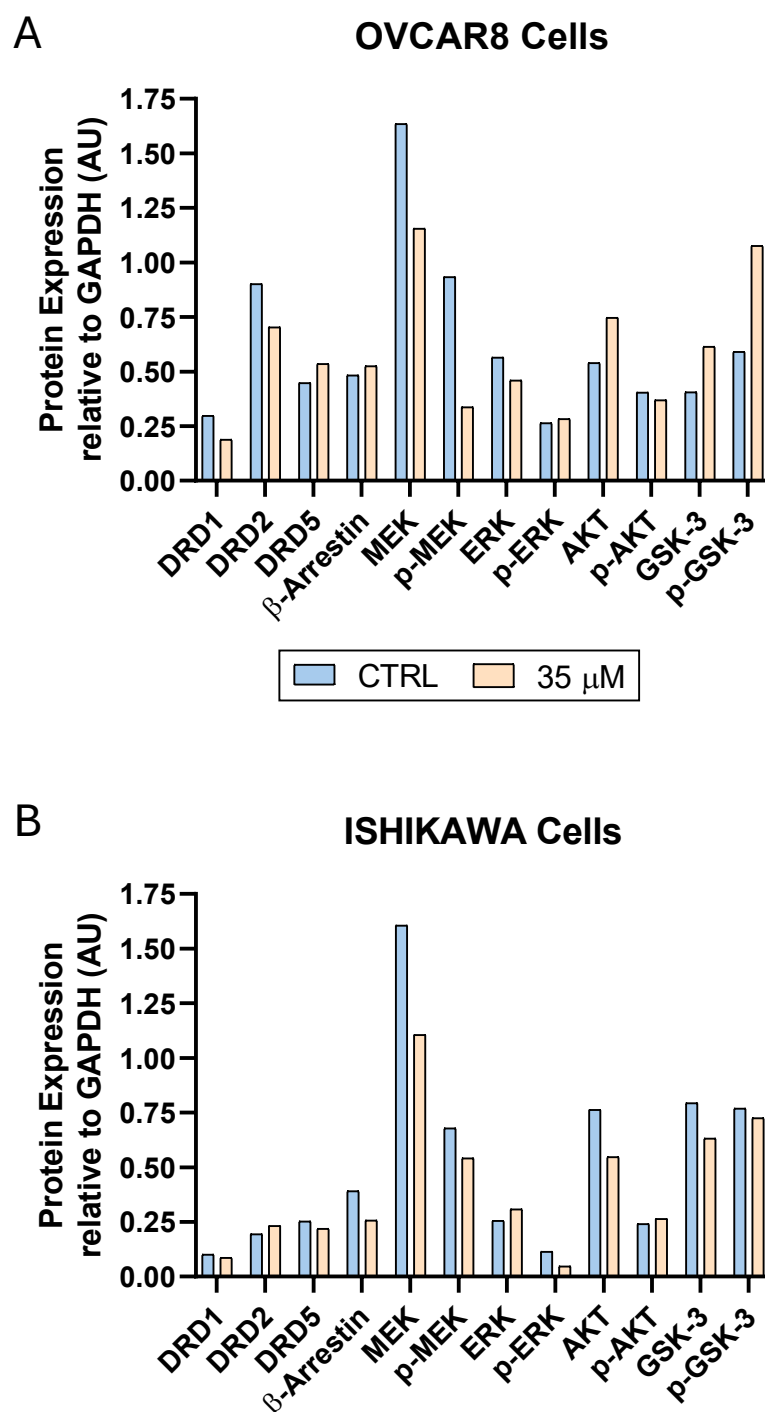


Figure 27. Effects of DRD2 antagonism (Domperidone) on protein expression in gynaecological cancer cell lines. The Western blot quantification of **(A)** ovarian cancer cell line (OVCAR8) and **(B)** endometrial cancer cell line (ISHIKAWA) treated with Domperidone (35 μ M) for 24h. Protein expression of DRD1, DRD2, DRD5, β -arrestin-2 and signalling molecules in the MEK/ERK (MAPK) and AKT/ GSK-3 signalling pathways were quantified by band intensity relative to GAPDH (AU) (n=1).

Chapter 4

The Efficacy of DRD2 Blocker

Domperidone in *ex vivo* Ovarian and
Endometrial OTSC Models and Patient-
Derived Biopsy Slices

Blocking DRD2 could be an advantageous anti-cancer strategy for gynaecological cancers. Recent publications have evidenced that DRD2 blockers have cytotoxic effects in *in vitro* and *in vivo* models of ovarian and endometrial cancer. Moreover, DRD2 antagonism could be beneficial in stimulating anti-tumour behaviours in immune cells. Given that gynaecological cancers are considered immunologically “cold” with depleted or immunosuppressed T cells and macrophages, blocking DR2-like receptors could be an efficacious strategy in the gynaecological TME.

Chapter 3 identified high expression of DRD2 in both ovarian and endometrial cancer models and highlighted the potent anti-cancer activity of the repurposed DRD2 blocker, Domperidone. As Domperidone is EMA-approved for the treatment of nausea, it could be a promising agent for drug repurposing, potentially avoiding lengthy, costly and risky drug approval pipelines. However, the translational gap between 2D cell culture and clinical application remains a primary cause of pharmaceutical failure. To bridge this gap, this chapter moves beyond simplistic monolayers to evaluate the efficacy of Domperidone within the complex, multi-component environment of the OTSC gynaecological model.

Chapter Aims

The primary objective of this chapter is to validate the therapeutic potential of Domperidone in a physiologically relevant environment. The specific aims are to:

- **Determine 3D Efficacy:** Assess the cytotoxic impact of Domperidone across established HGSOE and low-grade endometrial cancer OTSC models.
- **Characterise TME Modulation:** Investigate the spatiotemporal changes in cellular composition and signalling within the slice environment following treatment.
- **Validate in Patient Tissue:** Conduct a translational pilot study applying the OTSC pipeline to primary patient-derived ovarian cancer biopsies to evaluate individual treatment responses.

The findings from this chapter will provide a comprehensive characterisation of DRD2 blockade at both the cellular and molecular level, in physiologically relevant 3D OTSC gynaecological models and primary patient biopsy slices.

4.1 Methods

4.1.1 2D Monoculture Viability and Cytotoxicity Assays

For 2D monolayer cell line viability studies, cell lines were cultured in 24-well plates at 1×10^5 cells/well in 0.5 mL of appropriate media, grown for 24h (~70% confluency) and treated with Domperidone (70 μ M) or vehicle-only for 48h in standard cell culture conditions. Viability and cytotoxicity were measured using the AlamarBlue viability dye and CyQuant LDH kit as described before in Chapter 3.

4.1.2 OTSC and Real-Time and Fixed Time-Point Viability Assessments

ISHIKAWA and OVCAR8 whole tumours were implanted, resected, sliced and cultured according to our developed methodology in Chapter 3. After 24h of slice recovery, the media was replaced with fresh media containing 0, 35, 70, or 200 μ M Domperidone and incubated for 48h. Real-time (AlamarBlue and LDH) and fixed time point (IHC) assays were employed, as before, to assess viability and cytotoxicity after treatment using Ki67 and cleaved-caspase-3 staining (see Chapter 3).

4.1.3 Quantification of OTSC Slice Gene Expression using TLDA

Gene expression of OVCAR8 and ISHIKAWA xenografts OTSC tumour samples untreated or treated with 35 and 70 μ M Domperidone was performed by RT-qPCR using TaqMan probes specific for target genes. Probes and primers were selected for genes of interest, such as those related to catecholaminergic receptors, cancer cells, CSCs, immune and endothelial cell marker, and TaqMan Low Density Arrays (TLDA) cards (ThermoFisher) were manufactured. Each TLDA consists of 8 loading ports, allowing for simultaneous analysis of 24 genes in 8 samples (in duplicate) in a 384-well plate. In each well, 50 μ L of qPCR master-mix (SensiFAST™ ProbeHi-ROX kit; Biorline) and 50 μ L of cDNA (~1020 ng of total RNA) were added and reactions conducted in the 7900HT Fast Real Time PCR System (Applied Biosystems). Cycle thresholds (Ct) were measured and values calculated using the $2^{-\Delta Ct}$ method, normalised to the expression of the housekeeping gene GAPDH.

4.1.4 LIVE/DEAD Kit Staining and Confocal Microscopy

For further assessments of slice health following Domperidone incubation, the LIVE/DEAD™ Cytotoxicity/Viability kit (Invitrogen; L3224) was utilised to explore real-time viability and cytotoxicity markers simultaneously in the same slice. Slices were checked for viability on Day 0 and Day 1 using an AlamarBlue assay. Following Day 1 viability confirmation, slices were treated with vehicle or Domperidone (70 µM) for 48h. Following treatment, slices were transferred to a 96-well plate and washed three times in 100 µL PBS while rocking at 70 RPM within an incubator. A fluorescent viability mixture was prepared consisting of the kit reagents Ethidium homodimer-1 (2 µM), Calcein-AM (1 µM) and Hoechst 33342 (10 µg/mL), diluted in phenol red-free media to minimise background autofluorescence during confocal imaging. Ethidium homodimer-1 (Eth-1) serves as a high-affinity marker for cell death, as it is excluded by intact plasma membranes and only labels the nuclei of cells with compromised integrity. Whereas Calcein-AM functions as a marker for live cells, as it is converted to a green-fluorescent product by intracellular esterase activity within metabolically active cells with intact membranes. Hoechst 33342 acts as a cell-permeable counterstain to identify all nuclei within the 3D structure. A volume of 50 µL of this mixture is added to the slices for 1 hour. The slices were then washed three times in PBS and transferred to an imaging disc containing phenol red-free RPMI 1640 medium and imaged immediately on the Leica TCS SPE confocal microscope.

Images were processed using a custom ImageJ macro (Figure 28). Multichannel confocal images were split into individual 8-bit grayscale channels. To remove non-specific background noise, a rolling ball background subtraction (radius = 50 pixels) was applied to all channels. Fixed global thresholds were determined as: 120-255 pixels for blue (nucleus; Hoechst), 60-255 for green (alive cells; calcein-am), and 75-255 for Red (dead cells; Eth-1). Pixel area was calculated from the resulting masks. Percent viability was calculated as:

$$\%Viability = \frac{Area_{Green}}{(Area_{Green} + Area_{Red})} \times 100 \quad (8)$$

```

5  setBatchMode(true);
6
7  dir = getDirectory("Choose a Directory");
8  count = 0;
9  countFiles(dir);
10 n = 0;
11 processFiles(dir);
12
13 print("Batch Complete. " + n + " measurements recorded.");
14 setBatchMode(false);
15
16 function countFiles(dir) {
17     list = getFileList(dir);
18     for (i=0; i<list.length; i++) {
19         if (endsWith(list[i], "/")) countFiles(dir + list[i]);
20         else if (endsWith(list[i], ".tif")) count++;
21     }
22 }
23
24 function processFiles(dir) {
25     list = getFileList(dir);
26     for (i=0; i<list.length; i++) {
27         if (endsWith(list[i], "/")) processFiles(dir + list[i]);
28         else if (endsWith(list[i], ".tif")) {
29             showProgress(n++, count);
30             processFile(dir + list[i]);
31         }
32     }
33 }
34
35 function processFile(path) {
36     open(path);
37
38     depth = bitDepth();
39     getDimensions(width, height, channels, slices, frames);
40
41     if (channels > 1 || depth == 24) {
42         if (channels > 1 || depth == 24) {
43             run("Split Channels");
44         }
45         titles = getList("image.titles");
46         for (i=0; i<titles.length; i++) {
47             selectWindow(titles[i]);
48             currentTitle = getTitle();
49
50             // --- BLUE CHANNEL (DAPI) ---
51             if (indexOf(currentTitle, "blue") >= 0 || indexOf(currentTitle, "ch00") >= 0) {
52                 run("Subtract Background...", "rolling=50");
53                 setThreshold(120, 255);
54                 doMeasurement();
55             }
56             // --- GREEN CHANNEL (Live) ---
57             else if (indexOf(currentTitle, "green") >= 0 || indexOf(currentTitle, "ch01") >= 0) {
58                 run("Subtract Background...", "rolling=50");
59                 setThreshold(60, 255); // Updated to 60 as requested
60                 doMeasurement();
61             }
62             // --- RED CHANNEL (Dead) ---
63             else if (indexOf(currentTitle, "red") >= 0 || indexOf(currentTitle, "ch02") >= 0) {
64                 run("Subtract Background...", "rolling=50");
65                 setThreshold(75, 255); // Updated to 110 as requested
66                 doMeasurement();
67             }
68             else {
69                 close();
70             }
71         }
72     }
73 }
74
75 function doMeasurement() {
76     setOption("BlackBackground", true);
77     run("Convert to Mask", "method=Default background=Dark calculate black");
78     run("Create Selection");
79     run("Measure");
80     close();
81 }

```

Figure 28. Image J script for semi-automatised quantification of LIVE/DEAD confocal microscopy staining. Each image is separated by channels, the background is removed, the appropriate threshold is set and the staining area is quantified.

4.1.5 Flow Cytometry (Population Composition and Immune Subsets)

To evaluate the effects of Domperidone on specific immune populations within the slice, slices were treated with Domperidone (35 and 70 μ M) for 24h and 48h and disaggregated using our gentler disaggregation method e.g., 0.2 mg/mL TL Liberase and 10 μ g/mL DNase I for 1h in the Miltenyi GentleMACS dissociator. Slices were blocked with Fc receptor blocking (anti-CD16/CD32) and stained for dead cells (Viability™ Fixable Dye), as before (Chapter 2.1.13). The cell suspensions were stained for immune populations using 7-colour antibody mix (1:50) specific for CD45 (pan-immune), F4/80 (macrophage marker), CD3e (pan T lymphocyte marker), Ly-6G (neutrophil marker), CD19 (B cell marker), CD11c (dendritic cell marker), and NK1.1 (NK cell marker) (Table 21). Staining was performed for 20 min at 4 °C in the dark, with single-colour compensation controls prepared in parallel. After staining, cells were washed, fixed in 4% paraformaldehyde for 20 min at room temperature, resuspended in PBS/2% FBS, and stored at 4 °C in the dark until acquisition (within 1–2 days) with the MACSQuant® Analyzer 16.

Table 21. Immunofluorescent Antibodies used for flow cytometry of immune populations

Population	Marker	Clone	Fluorochrome	Commercial Source	Reference
Viable Cells	Viobility™ Fixable Dye	n/a	405/520	Miltenyi	130-130-404
Pan-leukocyte	CD45	REA737	VioBlue®	Miltenyi	130-110-802
Macrophages	F4/80	REA126	FITC	Miltenyi	130-117-509
T cells	CD3e	145-2C11	PE	Biolegend	100308
Neutrophils	Ly-6G	REA526	APC	Miltenyi	130-120-803
B cells	B220/CD19	REA749	PE-Vio615	Miltenyi	130-112-042
Dendritic Cells	CD11c	REA618	PE-Vio 770	Miltenyi	130-120-297
NK cells	NK1.1	REA1162	PerCP-vio700	Miltenyi	130-120-511

4.1.6 Patient-Derived OTSC of Ovarian Cancer Biopsy

To assess the efficacy of Domperidone in fresh human patient biopsy, 18 samples (OV5 – OV22) were resected and transported to our facilities for slicing and drug testing. Some samples were of inadequate quality and could not be successfully sectioned for downstream analysis. In total, 7 samples were processed and assessed in our OTSC evaluation system using AlamarBlue or CyQUANT LDH assays. One sample (OV7) was also evaluated for cell viability using the LIVE/DEAD immunofluorescence kit and confocal microscopy, as described in Chapter 2.

4.1.7 Survival Analysis of DRD2 Expression

To evaluate the effects of DRD2 gene expression on ovarian cancer, two primary datasets were used from The Cancer Genome Atlas (TCGA) and the Genome-Tissue Expression (GTEx) project. Differential expression between ovarian tumour and normal ovarian tissues was evaluated in GEPIA2 (<http://gepia2.cancer-pku.cn>) using the TCGA-OV cohort (n=426) and GTEx normal ovary samples (n=88). Boxplots were generated to compare median mRNA expression levels of DRD2 between tumour and normal tissues.

For survival analyses, overall survival (OS) was examined in GEPIA2 and OncoLnc (<http://www.oncolnc.org/>) using TCGA ovarian cancer data. Kaplan–Meier survival curves were generated for DRD2 gene. KMplot automatically selected the best cutoff value for group separation, and overall survival curves were generated with hazard ratios and log-rank p-values provided by the platform, the differences considered significant if $p < 0.05$.

4.1.8 Statistical Analysis

For 3-Day viability kinetics (Alamar Blue and LDH), IHC, TLDA, flow cytometry, patient OTSC and LIVE/DEAD data, statistical analyses were performed using linear mixed modelling using the lme4, car, and emmeans, lmerTest packages, with Slice ID as a random effect to account for the paired nature between control and treated slices, as well the models' ability to handle uneven sample sizes between conditions. Normality of residuals was assessed using Shapiro-Wilk tests and visual inspection of Q-Q plots, while homogeneity of variance with residual-versus-fitted plots. For datasets violating these assumptions, notably within the transcriptomics and IHC datasets, data were $\log_{10}$ transformed to stabilize variance and skew. Post-hoc testing was conducted either with Dunnett-style comparisons when evaluating treatments against a control reference only, or Tukey-adjusted pairwise comparisons for all-group analyses.

For comparisons of means normalised to the control (100%), one-sample t-tests were conducted and compared against a theoretical baseline of 100. Assumptions of the model such as normality and homogeneity of variances of the raw data were checked with Shapiro-Wilk and Q-Q plots. If assumptions failed non-parametric Wilcoxon signed-rank tests were employed. Specific dual-group comparisons were performed using Welch's unpaired t-tests or paired t-tests where appropriate e.g., 2D vs. 3D data.

4.2 Results

4.2.1 DRD2 Antagonism Significantly Reduces Viability in OVCAR8 OTSC Slices

OVCAR8 slices were produced using our validated protocol and treated with 35 or 70 μ M Domperidone for 48hr and changes in viability and cell death were evaluated as before, using the real-time and fixed assays. Results show that prior to treatment, all groups maintained excellent slice health on Day 1 and were not significantly different, with mean viabilities of 96.4% (control), 98.5% for 35 μ M treated slices (LMM, $p = 0.94$) and 98.8% for 70 μ M (LMM, $p = 0.98$). Control slices maintained this high viability reading until Day 3, exhibiting a slight increase to a value of 105.5%. In contrast, the Domperidone-treated slices showed significant dose-dependent decreases in viability on Day 3, with the 35 μ M treated slices exhibiting a mean viability of 65.6% (LMM, $p = 0.0035$) and 70 μ M treated slices with 40.28% (LMM, $p < 0.0001$). Moreover, 70 μ M treated slices were significantly less viable than the 35 μ M treatment (mean difference = 25.3 ± 4.21 , $p = 0.0023$), confirming the dose-dependent nature of the response (Figure 29A).

To assess the difference between matched treated slices and their biological controls, raw values were normalised by their internal controls. This data also shows a similar pattern; Domperidone at 70 μ M significantly reduced viability by 35.6% ($p = 0.0037$). Moreover, Domperidone at 200 μ M showed an even bigger impact, with a mean reduction in viability of 87.6%, however statistical analysis could not be conducted due to a small sample size ($n=2$). Whereas Domperidone at 35 μ M only showed a slight and non-significant reduction of 12.9% ($p = 0.460$) (Figure 29B). Similar findings were observed when comparing changes in proliferative Ki67 staining on Day 3 following 48h treatment with Domperidone, with analysis revealing a potent, significant suppression of proliferation in response to the drug (Figure 29C; Figure 30). Interestingly, treatment with 35 μ M Domperidone resulted in a pronounced reduction in Ki67 expression (mean = $24.7 \pm 11.1\%$) compared to control (100.0%), which was statistically significant ($t(2) = -14.57$, $p = 0.0047$). Similarly, treatment with 70 μ M Domperidone induced a significant reduction in the proliferation marker (mean = $32.2 \pm 24.8\%$) relative to controls ($t(2) = -5.88$, $p = 0.028$). The two treatment groups were not statistically different from one another in the IHC data set.

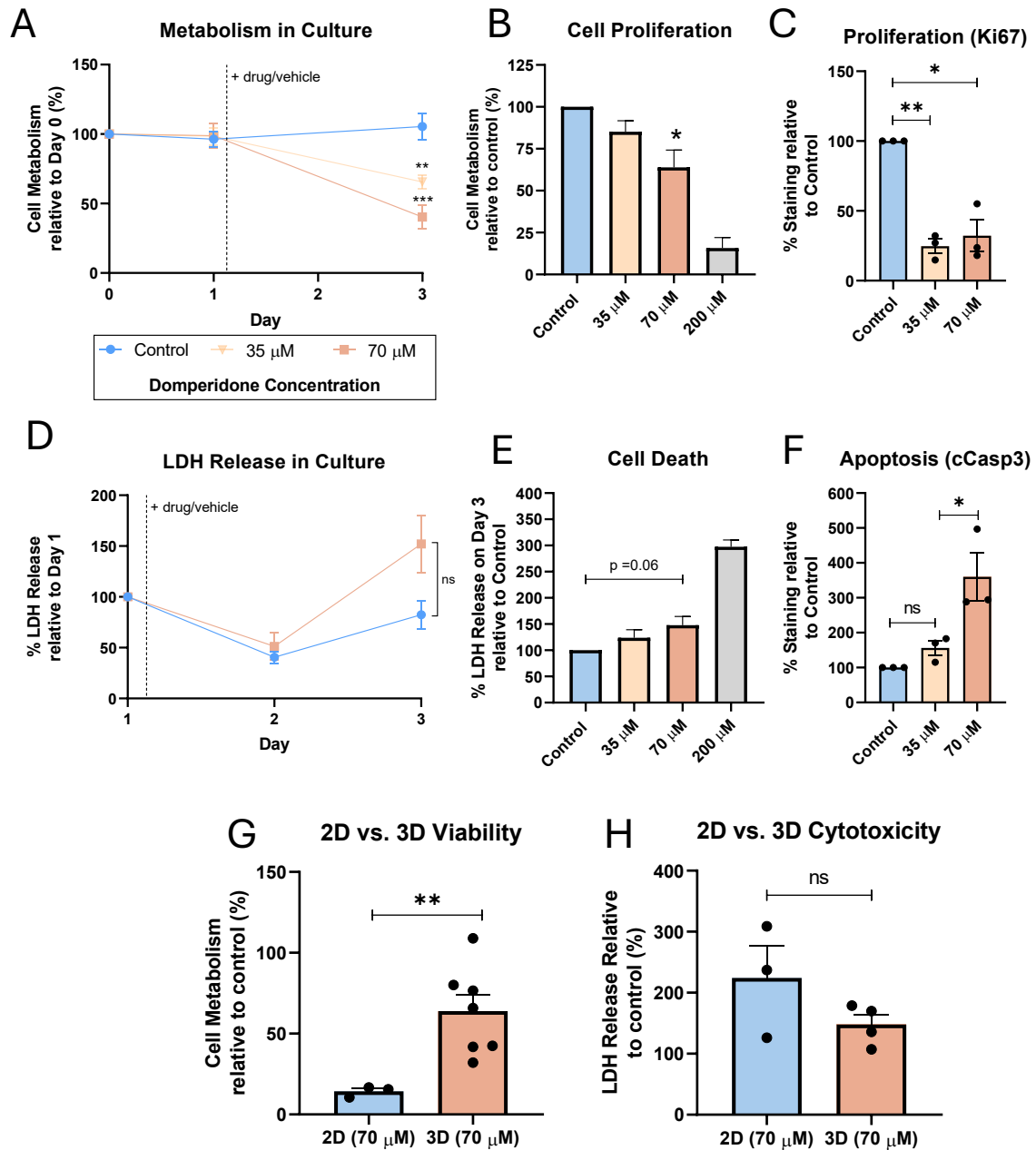


Figure 29. (A) Metabolic activity (AlamarBlue) of OVCAR8 tumour slices over a 3-day culture period ($n = 15$). Domperidone (35 μ M or 70 μ M) or vehicle was administered for 48h after 24h in culture. (B) Metabolic activity of treated slices on Day 3 expressed as a percentage of internal matched controls ($n = 6-7$). (C) Proliferation (Ki67) IHC staining intensity relative to control ($n = 3$). (D) Cell death (LDH release) in culture ($n = 6$). (E) Cytotoxicity (LDH release) on Day 3 normalised to control ($n = 3-4$). (F) Apoptosis (cleaved-Caspase 3; cCasp3) IHC staining relative to control ($n = 3$). (G-H) Comparison of Domperidone-induced cytotoxicity in 2D (cells) versus 3D (tumour slices): (G) Cell viability (AlamarBlue). (H) LDH release relative to corresponding vehicle controls. All data presented as mean $\pm$ SEM, performed in duplicate. Tested with Linear Mixed-Effects Model (A, D), One-Sample t -test (B, C, E, F), Wilcoxon Signed Rank (E), and Welch's t -test (G-H), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, ns = not significant.

In contrast, when comparing AlamarBlue viability between 2D cells and 3D slices, the 3D culture model exhibited a significantly higher metabolic signal of 64.0% compared to the 2D condition of 14.3% (Welch's t-test, $p = 0.0026$). This represents an approximately 4.5-fold increase in metabolic activity in the 3D model, confirming that the monolayer cultures are more sensitive to 70 μ M Domperidone than the slices (Figure 29G).

4.2.2 DRD2 Antagonism Increases Apoptosis in OVCAR8 OTSC Slices

To compare the cytotoxic response of the slices during treatment with Domperidone, LDH release was measured on Day 1 (prior to treatment), then again after 24h (Day 2) and 48h (Day 3) incubation with 35 and 70 μ M Domperidone. Following 24h with vehicle or treatment, both groups displayed a reduction in LDH release, with control releasing 59.4% less LDH and the treatment group 49.8% less compared to their day 0 baseline, however these values were not statistically different ($p = 0.386$). These results suggest that significant cytotoxicity had not yet manifested by 24h of treatment (Figure 29D).

On Day 3, both groups experienced a rise in LDH release (Figure 29D). While the control still displayed less cytotoxicity than Day 1 with a mean value of 82.5%, the treatment group was almost 2-fold higher with 152.1% LDH release. Global analysis of the data indicated a significant effect of treatment group ($\chi^2(1) = 10.04$, $p = 0.0015$). However, post-hoc comparisons (Kenward-Roger method) revealed that this difference approached but did not reach statistical significance ($p = 0.087$).

Normalised data also revealed a trend toward increased cytotoxicity with increasing drug concentration (Figure 29E). Treatment with 35 μ M Domperidone resulted in a slight, non-significant increase in LDH release (Mean = $123.9 \pm 26.6\%$) relative to control ($t(2) = 1.56$, $p = 0.260$). In contrast, treatment with 70 μ M Domperidone elicited a more pronounced response but again did not reach statistical significance relative to the control baseline ($t(3) = 2.91$, $p = 0.062$). The pattern of increasing LDH release levels continued with 200 μ M with a mean LDH release of 297.5% ($\pm 18.6\%$) compared to the Control, however statistical testing could not be conducted due to the small sample size ($n=2$).

When examining apoptotic marker staining (cleaved-caspase-3) in the Day 3 slices, treatment with 35 μ M Domperidone resulted in a modest increase in signal (Mean = 156.3 $\pm$ 71.8%) compared to control, but this difference was not statistically significant ($t(2) = 2.72$, $p = 0.113$). In contrast, treatment with 70 μ M Domperidone induced a massive, 3.6-fold increase in the death marker (Mean = 359.7 $\pm$ 237.0%) compared to controls (Figure 29F; Figure 30). However, this increase did not reach statistical significance relative to the control baseline ($t(2) = 3.80$, $p = 0.063$). However, a direct comparison between the two Domperidone treatment groups (Two-Sample t-test) confirmed that the apoptotic signal was significantly higher in the 70 μ M treated slices compared to the 35 μ M dose ($t(4) = -2.85$, $p = 0.047$), verifying a dose-dependent toxic effect. Similarly, the 2D monolayers exhibited a higher mean LDH release (224.0%) compared to the 3D slices (148.0%), however statistical analysis revealed no significant difference in LDH release between the two models ($df = 2.39$, $t = 1.37$, $p = 0.286$) (Figure 29H).

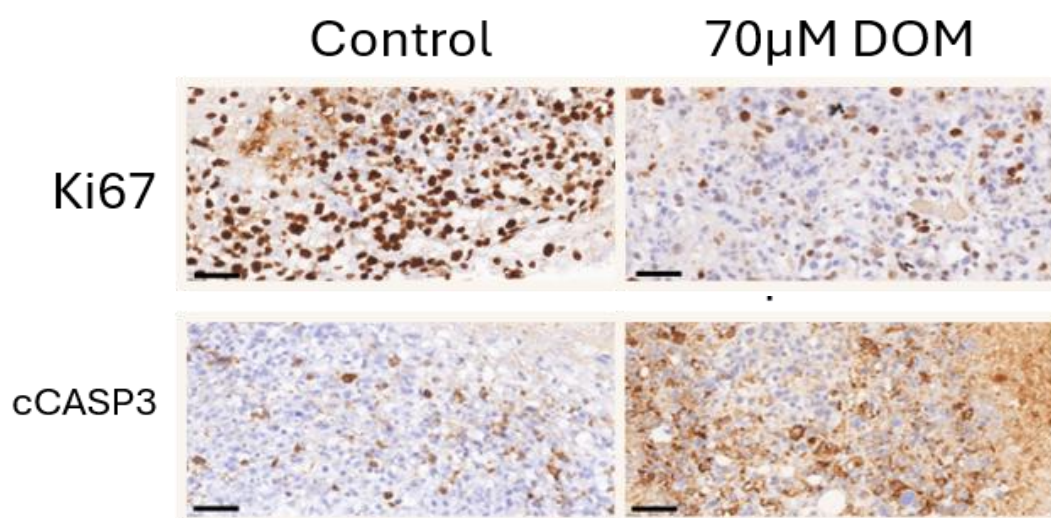


Figure 30. Microscopy images of OVCAR8 slices treated with or without Domperidone (DOM) and stained with Ki67 (proliferation; above) or cleaved-caspase-3 (cCASP3; apoptosis; below) specific DAB antibodies. Scale bar = 50 μ m.

4.2.3 Endometrial OTSC Model Exhibits Potent Resistance to Domperidone

To investigate the temporal impact of Domperidone at 70 μ M on ISHIKAWA tissue health, AlamarBlue viability was monitored longitudinally at Day 0, 24 hours after culture (Day 1), and then again 48 hours (Day 3) post-treatment compared to baseline (Day 0 = 100%). In the stabilisation phase (24 hours), no significant difference in viability was observed between the groups ($\chi^2(1) = 0.07$, $p = 0.786$). The estimated mean AlamarBlue viability for the control group was 76.9% ($\pm 7.0\%$ SE), while the 70 μ M treatment group exhibited a comparable mean of 78.2% ($\pm 6.6\%$ SE). Post-hoc pairwise comparison confirmed that the 1.3% difference was not statistically significant ($p = 0.807$), suggesting that both groups had the same baseline health prior to treatment (Figure 31A).

Following 48 hours of exposure, a highly significant treatment effect was detected ($\chi^2(1) = 31.65$, $p < 0.0001$). Mean AlamarBlue viability in the 70 μ M group rose to 94.3% ($\pm 9.1\%$ SE) compared to 65.9% ($\pm 9.4\%$ SE) in the control group. Post-hoc analysis revealed that the 70 μ M treatment significantly increased viability by a mean of 28.4% above control levels ($p = 0.0108$) (Figure 31A). However, differences did not reach significance when analysing control-normalised data, likely due to the reduced power of the statistical one-sample t-test ($t(4) = 1.94$, $p = 0.125$) (Figure 31B). This metabolic increase is also vastly different to the results of 2D testing, where the 2D monolayer had significantly lower metabolic signal of a mean of 19.3% ($\pm 2.9\%$ SD) compared to the 3D condition of 135.7% ($\pm 44.1\%$ SD) ($df=4.06$, $t = -6.05$, $p = 0.0036$). This represents a 7-fold increase in metabolic activity in the 3D model, confirming that the slices retain significantly higher metabolic output than standard monolayer cultures (Figure 31G). Analysis of Ki67 staining intensity ($n=4$) supports a lack of effect of 70 μ M Domperidone which resulted in a mean proliferative staining of $89.9 \pm 26.2\%$ relative to vehicle control (100%). This slight reduction was not statistically significant ($t(3) = -0.77$, $p = 0.498$), indicating that the drug did not effectively suppress cell division (Figure 31C; Figure 32).

LDH release was also monitored longitudinally after 24 hours (Day 2) and 48 hours (Day 3) post-treatment compared to baseline (Day 1 = 100%). In the initial phase (Day 2), no significant difference in LDH release was observed between the groups ($\chi^2(1) = 0.44$, $p = 0.506$). The estimated mean LDH release for the control group was 72.7% ($\pm 32.8\%$ SE), while the 70 μ M treatment group exhibited a mean of 85.8% ($\pm 32.8\%$ SE). Post-hoc

pairwise comparison confirmed that the 13.1% difference was not statistically significant ($p = 0.575$), indicating comparable tissue health between groups despite 24 hours of treatment (Figure 31D). After 48 hours exposure, the treatment group exhibited a marked increase in mean LDH leakage, although the overall effect did not reach statistical significance in this cohort ($\chi^2(1) = 1.21$, $p = 0.272$). Mean LDH release in the 70 μM group rose to 213.0% ($\pm 75.4\%$ SE) compared to 116.0% ($\pm 75.4\%$ SE) in the control group. Post-hoc analysis revealed a mean estimate difference of 97.3% between groups ($p = 0.3862$). Similarly, with the normalised data, treatment with 70 μM Domperidone induced a substantial increase in cytotoxicity, with mean LDH release rising to $228.8 \pm 76.4\%$ relative to control (Figure 31E). However, due to the variability inherent in the small sample size, this >2-fold increase did not reach statistical significance ($t(2) = 2.92$, $p = 0.100$). Apoptotic signalling was also assessed in a separate cohort ($n=4$). Unexpectedly, treatment with 70 μM Domperidone resulted in a trend towards reduced apoptotic signalling compared to the control baseline. The mean signal for the treatment group was $53.5 \pm 38.1\%$, representing a nearly 50% reduction in the apoptosis marker. However, difference did not reach statistical significance ($t(3) = -2.44$, $p = 0.092$) (Figure 32).

To demonstrate differences in drug sensitivity of the 2D and 3D models, the two systems were treated with 70 μM Domperidone for 48h and LDH release fold-change was calculated compared to their internal controls (2D or 3D models treated with vehicle only). The normalised metabolic changes of 3D slices ($n=5$) were compared to normalised 2D monolayers ($n=3$). The 3D slices exhibited a higher mean LDH release of 228.8% compared to 148.6% in the 2D monolayers, but the difference did not reach statistical significance ($df = 2.19$, $t = -1.78$, $p = 0.207$) (Figure 31H).

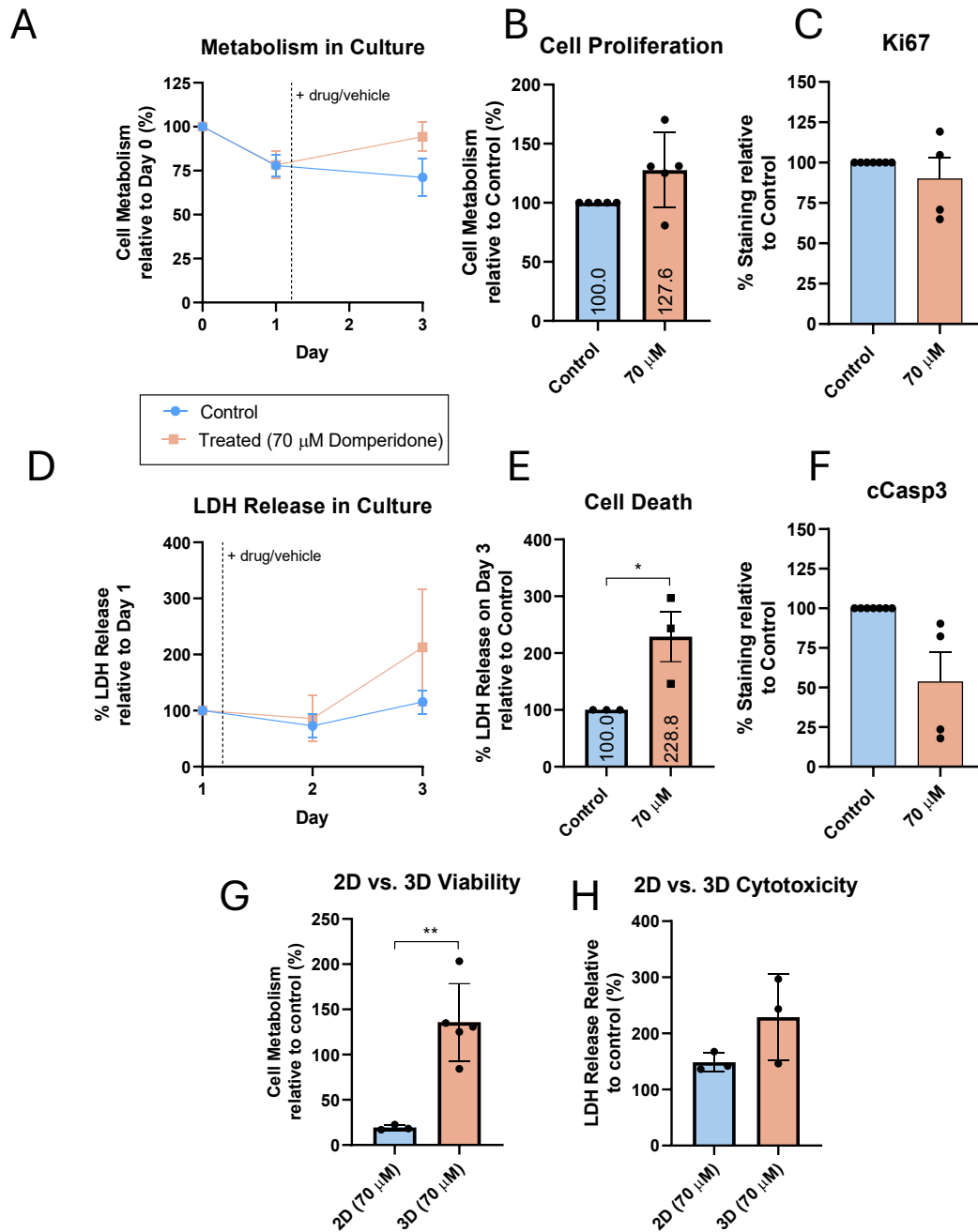


Figure 31. (A) Metabolic activity (AlamarBlue) of ISHIKAWA tumour slices over a 3-day culture period. Domperidone (70 μ M) or vehicle were administered for 48h after 24h in culture (dotted line). (B) Metabolic activity of treated slices on Day 3 normalised to control ($n = 5$). (C) Proliferation (Ki67) IHC staining intensity relative to control ($n = 4$). (D) Cell death (LDH release) in culture ($n = 3-4$). (E) Cytotoxicity (LDH release) of treated slices on Day 3 relative to control ($n = 3$). (F) Apoptosis (cleaved-Caspase 3; cCasp3) IHC staining relative to control ($n = 4$). (G-H) Comparison of Domperidone-induced cytotoxicity in 2D (cells) versus 3D (tumour slices): (G) Cell viability (AlamarBlue; $n = 3-5$), (H) LDH release relative to corresponding vehicle controls ($n = 3-5$). Data presented as mean $\pm$ SEM, in duplicate. Tested with Linear Mixed-Effects Modelling (A, D), One-Sample t-test (B, C, E, F), Wilcoxon Signed Rank (E), and Welch's t-test (G-H), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, ns = not significant.

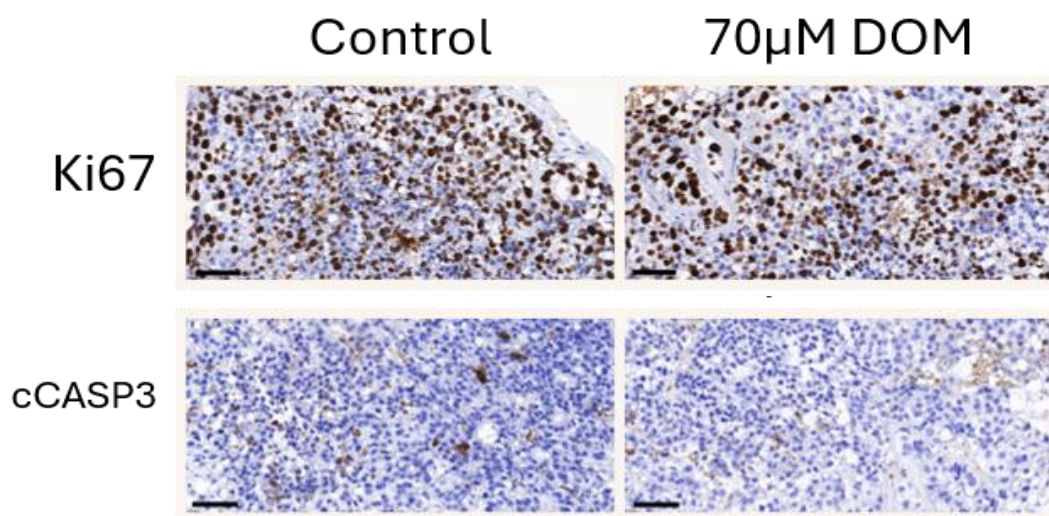


Figure 32. Microscopy images of ISHIKAWA slices treated with or without Domperidone (DOM) and stained with Ki67 (proliferation; above) or cleaved-caspase-3 (cCASP3; apoptosis; below) specific DAB antibodies. Scale bar = 50 μ m.

4.2.4 Confocal Assessment Confirms Selective Cell Death Following DRD2 Blockade

Viability of OVCAR8 slices were assessed using Live/Dead confocal microscopy following treatment with either 35 μ M Domperidone, 70 μ M Domperidone or chemotherapy (5 μ M Taxol/ 300 μ M Carboplatin) (Figure 33) and ISHIKAWA slices with 70 μ M Domperidone (Figure 34), compared with media-only control slices. Images were then quantified by calculating the area of alive cells as a proportion of the total area of both dead and alive cells.

For OVCAR8 slices, image analysis revealed a clear dose-dependent reduction in cell viability. The control group exhibited high viability after 3 days in culture (media-only) with a mean value of 94.8%. In contrast, treatment with 35 μ M Domperidone resulted in a decrease in the proportion of live cells to 74.6% ($\pm$ 6.74), however, linear mixed effect modelling with Tukey post hoc analysis revealed this reduction did not reach statistical significance ($p = 0.101$). In contrast, the 70 μ M treatment group showed a strong and highly significant reduction in viability to 48.2% ($\pm$ 7.38) compared to the control ($p <$

0.0001). Moreover, the higher concentration also achieved a significantly greater reduction in the proportion of alive cells than the lower 35 μ M Domperidone condition ($p = 0.0261$). While treatment with 70 μ M Domperidone also caused a greater viability loss than the chemotherapy-treated group ($73.6\% \pm 14.0$), this did not reach statistical significance ($p = 0.365$) (Figure 33A-B). However, it should be noted that the chemotherapy group consisted of very few samples ($n=3$) compared to the other groups ($n=12-15$). In contrast, ISHIKAWA slices treated with 70 μ M Domperidone did not achieve a significant loss in viability. In fact, as seen in Figure 34B-C, very few dead cells were observed at all. Compared to the control slices which had a mean viability of 88.6% at the end of the experiment, the treated group had a small non-significant viability loss, with a mean value of 78.72% ($p = 0.118$) (Figure 34A).

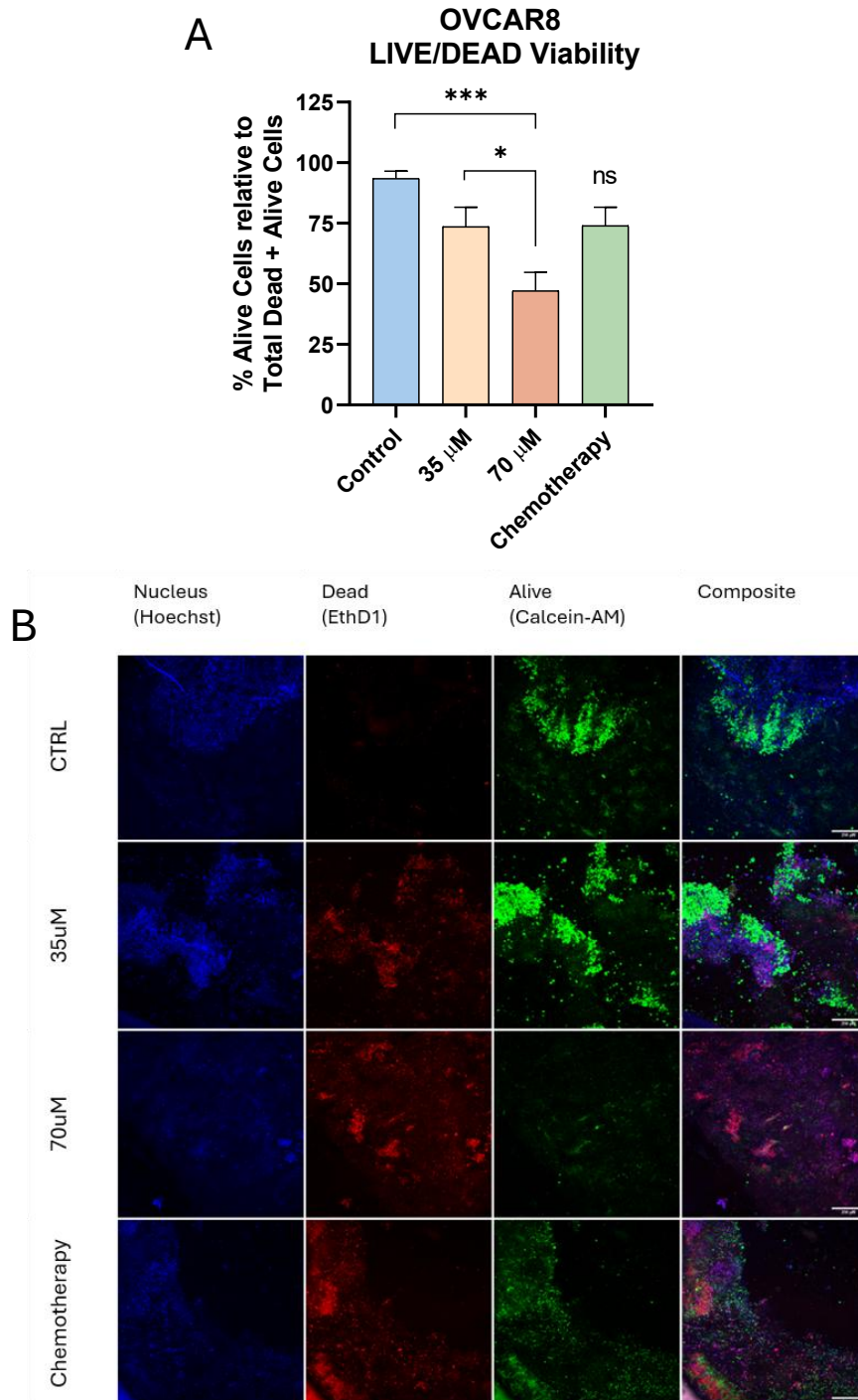


Figure 33. LIVE/DEAD staining of OVCAR8 tumour slices. (A) Quantification of OVCAR8 slices treated with media-only (CTRL), Domperidone (35 or 70 μ M) or Chemotherapy (5 μ M Taxol/ 300 μ M Carboplatin) for 48h. Slices were stained with LIVE/DEAD kit, imaged with confocal microscopy (representative images shown in B) and calculated as area of alive cells (calcein-AM; green) as a percentage of total alive + dead cells (Ethidium homodimer-1). Data presented as mean $\pm$ SEM (n=5). Statistical significance determined using a linear mixed-effects model (LMM) followed by Tukey's post-hoc test for pairwise comparisons. * p <0.05, *** p <0.0001, ns = not significant. Scale bar = 250 μ m.

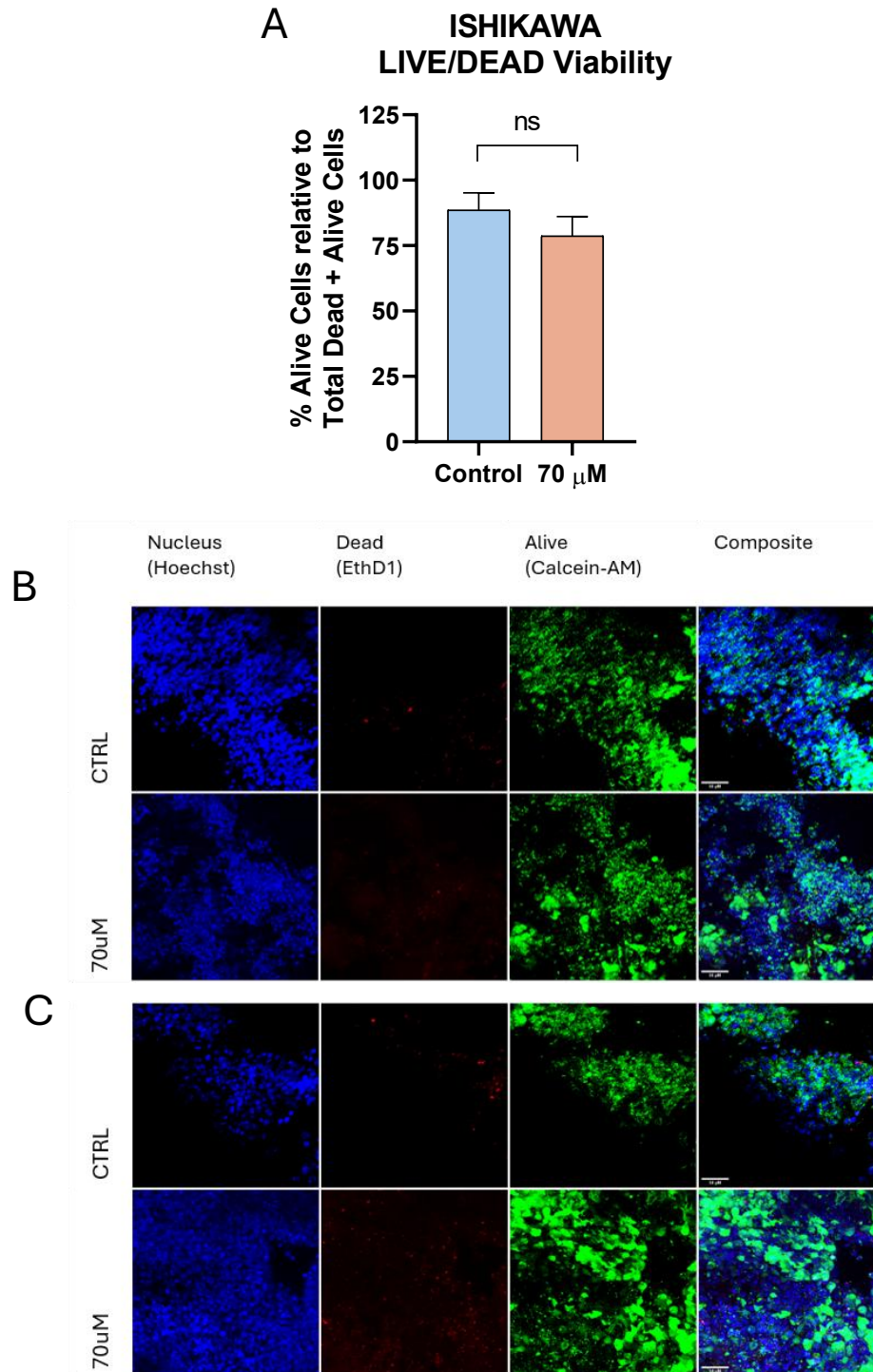


Figure 34. LIVE/DEAD staining of ISHIKAWA tumour slices. (A) Quantification of ISHIKAWA slices treated with either media-only (CTRL) or 70 μ M for 48h. Slices were stained with LIVE/DEAD kit, imaged with confocal microscopy (representative images shown in B-C) and calculated as area of alive cells (calcein-AM; green) as a percentage of total area of alive + dead cells (Ethidium homodimer-1; red). Data presented as mean $\pm$ SEM (n=3). Statistical significance determined using a linear mixed-effects model (LMM) followed by Tukey's post-hoc test for pairwise comparisons, ns = not significant. Scale bar = 50 μ m.

4.2.5 DRD2 Inhibition Differentially Modulates Immune Subsets in OTSC Models

For OVCAR8 xenografted tumour slices, at 24 hours, the tissue composition remained stable. Despite an observable decline in EPCAM+ cells with increasing Domperidone concentrations, statistical analysis detected no statistically significant differences between the Control, 35 μ M, and 70 μ M groups for the cancer cell fraction. Similarly, no differences were detected for immune (CD45+) or vascular (CD31+) fractions (Figure 35A-B).

However, after 48 hours of treatment, significant differences in population abundance were noted (Figure 35C). Firstly, for CD31+ endothelial cells, there was a significant main effect of treatment ($\chi^2 = 10.03$, $p = 0.007$). Post-hoc analysis confirmed that the 70 μ M treatment significantly increased the frequency of CD31+ cells compared to the Control (0.65 vs. 0.41, LMM, $p = 0.036$). While there was also an increase in CD31+ cells between the two treatment concentrations, with 35 μ M treated slices containing approximately 0.58% CD31+ cells and 70 μ M treated slices with 0.65%, this difference was not statistically different ($p = 0.659$). Similarly, for CD45+ immune cells there was also a significant main effect of treatment ($\chi^2 = 0.78$, $p = 0.005$). However, post-hoc analysis confirmed that this was driven by differences between the treatment groups in which the lower dosage group (35 μ M) had approximately 14.8% CD45+ cells and 70 μ M treated slices had a significant increase with 18.1% ($p = 0.0279$). Whereas the Control contained on average 16.1% immune cells, and this did not reach statistical significance in comparison to both the 70 μ M group ($p = 0.17$) and the 35 μ M group ($p = 0.454$). Interestingly, there was no main effect of treatment on the abundance of the epithelial cancer cell marker EpCAM ($p = 0.345$) or on the number of DRD2 positive which, although displayed a decrease between the control and higher dosage (1.37% vs. 1.16%), no main effect was detected ($p = 0.326$).

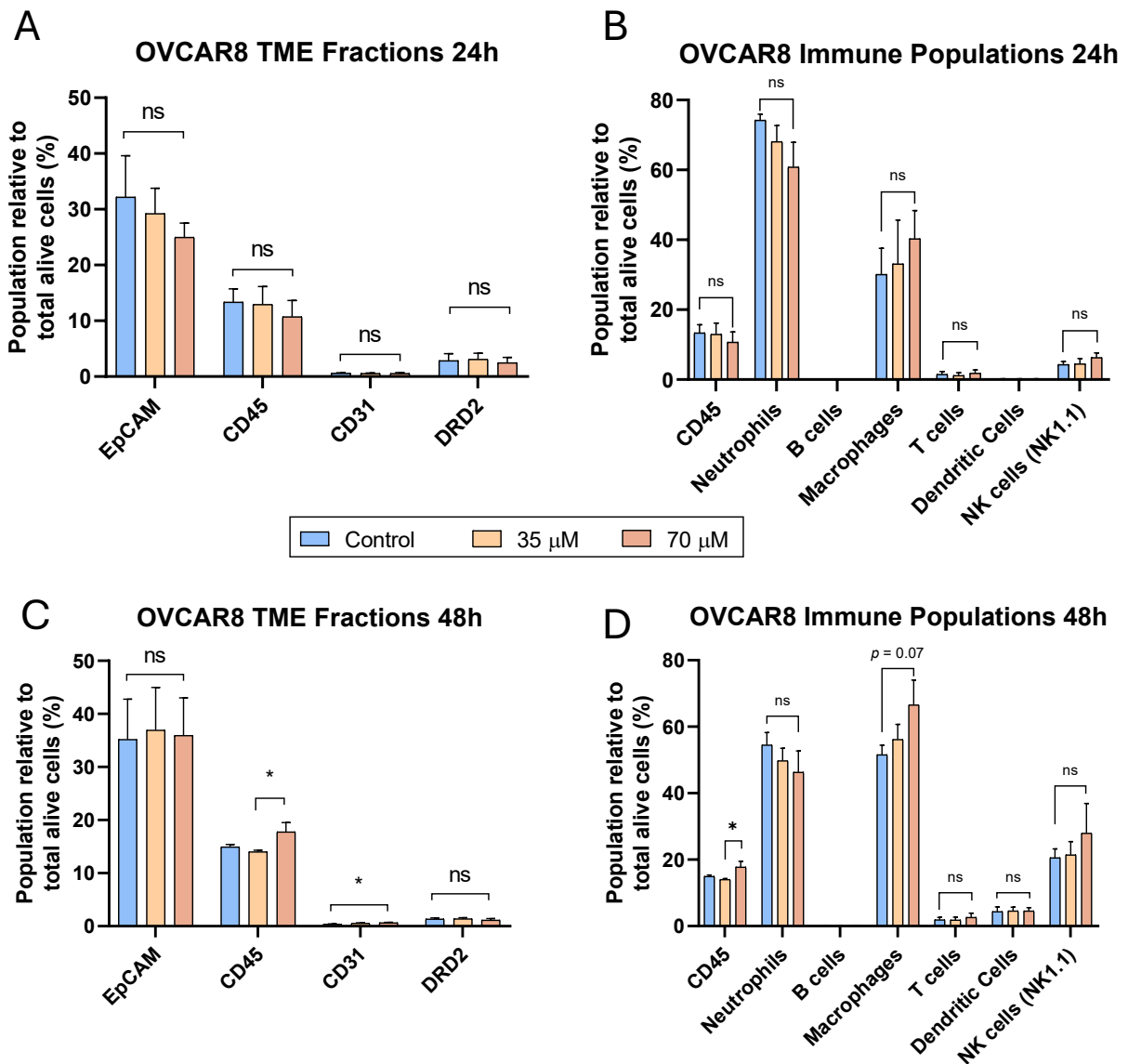


Figure 35. Temporal response of cell populations within OVCAR8 xenografted tumour slices to Domperidone treatment. (A-D) Cell population percentages within disaggregated OVCAR8 tumour slices treated with 35 μ M or 70 μ M Domperidone for 24 and 48 hours and measured by flow cytometry. (A) Quantification of cell fractions with immunofluorescent antibodies specific for cancer (EpCAM+), immune (CD45+), and vascular (CD31+) markers at 24h and (C) 48h. (B) Quantification of CD45+ immune subpopulations at 24h and (D) 48h. Statistical significance was determined using Linear Mixed Models (LMM) and post-hoc pairwise comparisons. Data are presented as mean percentages $\pm$ SE ($n=3-5$). Significance levels: * $p < 0.05$, ns = not significant.

When looking at the individual CD45+ populations (e.g., B cells, Dendritic cells, Macrophages, NK cells, Neutrophils and T cells), initially, three patterns were observed for both 24h and 48h, in which it appeared that neutrophils were declining, and macrophages and NK cells were increasing in abundance with rising Domperidone doses compared to the Control. However, further analysis into the 24h Control and Domperidone drug conditions revealed no statistically significant main effects for any of the immune cell populations (Figure 35B). Moreover, for the 48h treated slices, while a significant main effect was observed for macrophages ($p = 0.0264$), post hoc analysis revealed that compared to the Control group (51.6%), mean percentages in 35 μ M (51.6%, $p = 0.222$) and 70 μ M (66.6%, $p = 0.0697$) treatment groups were not significantly different. Furthermore, no significant main effect between Control and Treatment groups was revealed for mean NK cell percentages ($p=0.598$), and similarly for Neutrophils, main effect was just shy of reaching significance ($p = 0.0506$) (Figure 35D).

In contrast, ISHIKAWA xenografted slices treated with Domperidone for 24h or 48h did not cause any significant changes in global population composition, with all three major populations remaining highly comparable to the control group, even at the highest Domperidone concentration (CD45: $p = 0.75$, EpCAM: $p = 0.61$, CD31: $p = 0.11$) (Figure 36A, C). However, it should be noted that population size in the 48h groups was very small ($n=3$) and one outlier for each group had to be removed for CD45+ cells, in which slices from the same biological subject contained almost no CD45+ cells ($<1\%$). However, cytometry analysis of immune subsets revealed a stark comparison to OVCAR8 treated slices, indicating a distinct temporal and dose-dependent immune response (Figure 36B). After 24-hour treatment, particularly at 70 μ M, a significant increase of innate immune cells such as Neutrophils, Macrophages, and NK cells was observed (Table 22).

Table 22. Immune population changes in ISHIKAWA OTSC slices treated with Domperidone 70 μ M for 24h.

Cell Type	Control Mean	70 μ M Mean	Difference	Adjusted p-value
Neutrophils	7.28	17.90	+10.63	0.0065
NK Cells	24.60	45.90	+21.26	0.0064
Macrophages	47.10	75.00	+28.00	0.0271

In contrast, by 48 hours, these effects appear to have resolved or diminished, with no statistically significant differences observed between treatment and control groups in pairwise comparisons (Figure 36D). However, global significant dose-dependent trends were observed with decreasing abundance of B cells ($p < 0.001$) and T cells ($p < 0.001$) as the concentration of Domperidone increased. However, pairwise comparisons did not reveal any significant difference between specific sub-groups. Interestingly, it appeared that for all groups, abundance of macrophages relative to the total alive population was especially high in the range of 96-99.2%, and minor increases were observed in a dose-dependent manner compared the control ($p < 0.001$), yet group-specific significant differences were not observed.

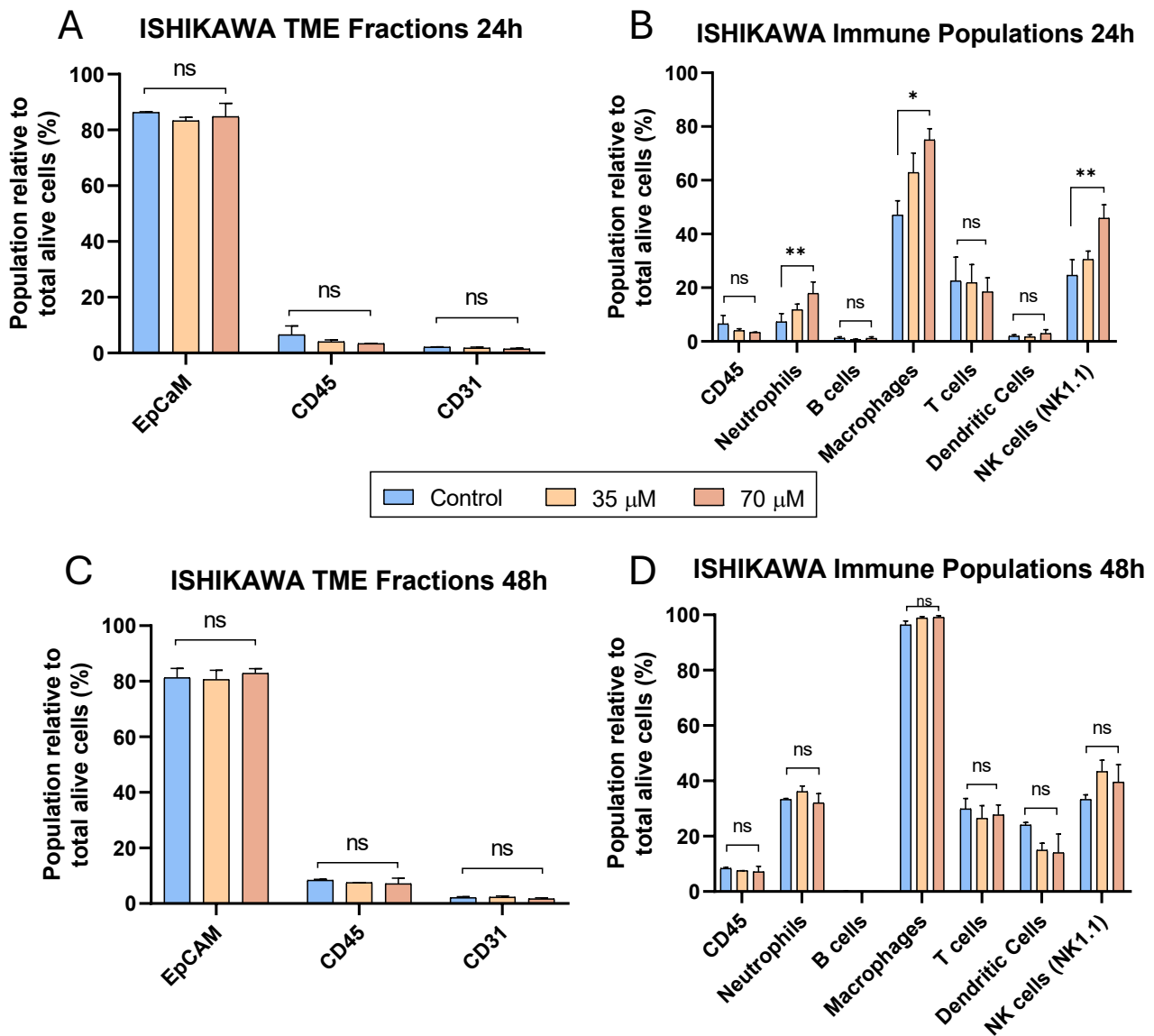


Figure 36. Temporal response of cell populations to Domperidone treatment within ISHIKAWA xenografted tumour slices. (A-D) Cell population percentages within disaggregated OVCAR8 tumour slices treated with 35 μ M or 70 μ M Domperidone for 24 and 48 hours and measured by flow cytometry. **(A)** Quantification of cell fractions with immunofluorescent antibodies specific for cancer (EpCAM+), immune (CD45+), and endothelial cell (CD31+) markers at 24h and **(C)** 48h. **(B)** Quantification of CD45+ immune subpopulations at 24h and **(D)** 48h. Statistical significance was determined using Linear Mixed Models (LMM) and post-hoc pairwise comparisons. Data are presented as mean percentages $\pm$ SE ($n=3-5$). Significance levels: * $p < 0.05$, ns = not significant.

4.2.6 DRD2 Blockade Upregulates the Transcription of Stemness Genes

To assess changes in gene expression following 48h treatment with Domperidone compared with vehicle-only, the slices were disaggregated and the RNA extracted and quantified using RT-qPCR. Given that the tumours were derived from xenografted mouse models, the resulting cDNA was analysed with probes specific for both human and mouse genes.

Analysis revealed dose-dependent changes in gene expression upon treatment with Domperidone, causing the upregulation of 8 human genes (Figure 37A). Four of these genes were upregulated with both high and low concentrations of Domperidone. This included CD24 (35 μ M: $p = <0.0001$, 70 μ M: $p = <0.0001$), CD44 (35 μ M: $p = 0.0025$, 70 μ M: $p = <0.0001$), EPCAM (35 μ M: $p = 0.0116$, 70 μ M: $p = 0.0006$), and PROM1 (35 μ M: $p = 0.0137$, 70 μ M: $p = 0.0017$) (Table 23). Whereas for the other four genes, only 70 μ M caused a significant upregulation, for instance ADRA1A ($p = 0.0019$), DRD1 ($p = 0.0031$), DRD5 ($p = 0.0054$), and CXCR4 ($p < 0.0001$) compared to vehicle controls (Table 23). It should be noted that during statistical analysis, DRD1, DRD5, and CXCR4 underwent a log-transformation to address significant heteroscedasticity. While these targets retained a degree of non-normality after transformation (Shapiro-Wilk $p < 0.05$), residual plots demonstrated normality. Interestingly, ADRA1A, CXCR4 and DRD1 and DRD5 expression increased by approximately 10-fold in slices treated with 70 μ M Domperidone in comparison to the Control group. Whereas CD24, CD44 and EpCAM expression increased by roughly two-fold. In contrast, Domperidone treatment did not cause a significant change in expression of ACKR3, ADRA1B, ALDH1A1 and DRD2.

For mouse-specific genes, expression of *DRD1* was significantly elevated in the OVCAR8 70 μ M group (0.00806) compared to the Control group (0.00400, $p = 0.0171$) (Figure 37B). Whereas expression in the 35 μ M group (0.00729, $p = 0.0847$) did not differ significantly from the Control (Table 24). In contrast, *Pecam1* that encodes the endothelial cell marker CD31 was significantly downregulated upon treatment with 70 μ M (mean = 0.00078) compared to the control (mean = 0.00648, $p = 0.0022$). Similarly, expression of Ly6c1;Ly6c2 encoding the macrophage and monocyte specific receptor also significantly decreased from 0.00592 in the Control group to 0.00123 ($p = 0.0055$) in the 70 μ M Domperidone condition but did not reach statistical significance in the 35 μ M group.

While expression of Ptpcr encoding the pan-leukocyte CD45 protein appeared to decrease with increasing Domperidone concentration, statistical analysis did not identify any significant changes. Similarly, no significant changes were detected for expression of Adre1, Itga2, Itgam and DRD5 (Figure 37B).

Table 23. Summary of statistically significant changes in expression of human-specific genes in OVCAR8 slices treated with 35 and 70 μ M Domperidone for 48h.

Gene	Control	35 μ M	p-value	70 μ M	p-value
ADRA1A	0.00198	0.00480	0.2187	0.0222	0.0019
CD24	0.0195	0.0345	<0.0001	0.0487	<0.0001
CD44	0.0183	0.0283	0.0025	0.0300	<0.0001
CXCR4	0.00177	0.00435	0.0565	0.0117	<0.0001
DRD1	0.00197	0.00383	0.3601	0.0205	0.0031
DRD5	0.00206	0.00408	0.4103	0.0211	0.0054
EPCAM	0.00333	0.00542	0.0116	0.00583	0.0006
PROM1	0.00002	0.00007	0.0137	0.00008	0.0017

Table 24. Summary of statistically significant changes in expression of mouse-specific genes in OVCAR8 slices treated with 35 and 70 μ M Domperidone for 48h.

Gene	Control	35 μ M	p-value	70 μ M	p-value
DRD1	0.00400	0.00729	0.0847	0.00806	0.0171
LY6C1;LY6C2	0.00592	0.00173	0.2819	0.00123	0.0055
PECAM1	0.00648	0.00217	0.2949	0.00078	0.0022

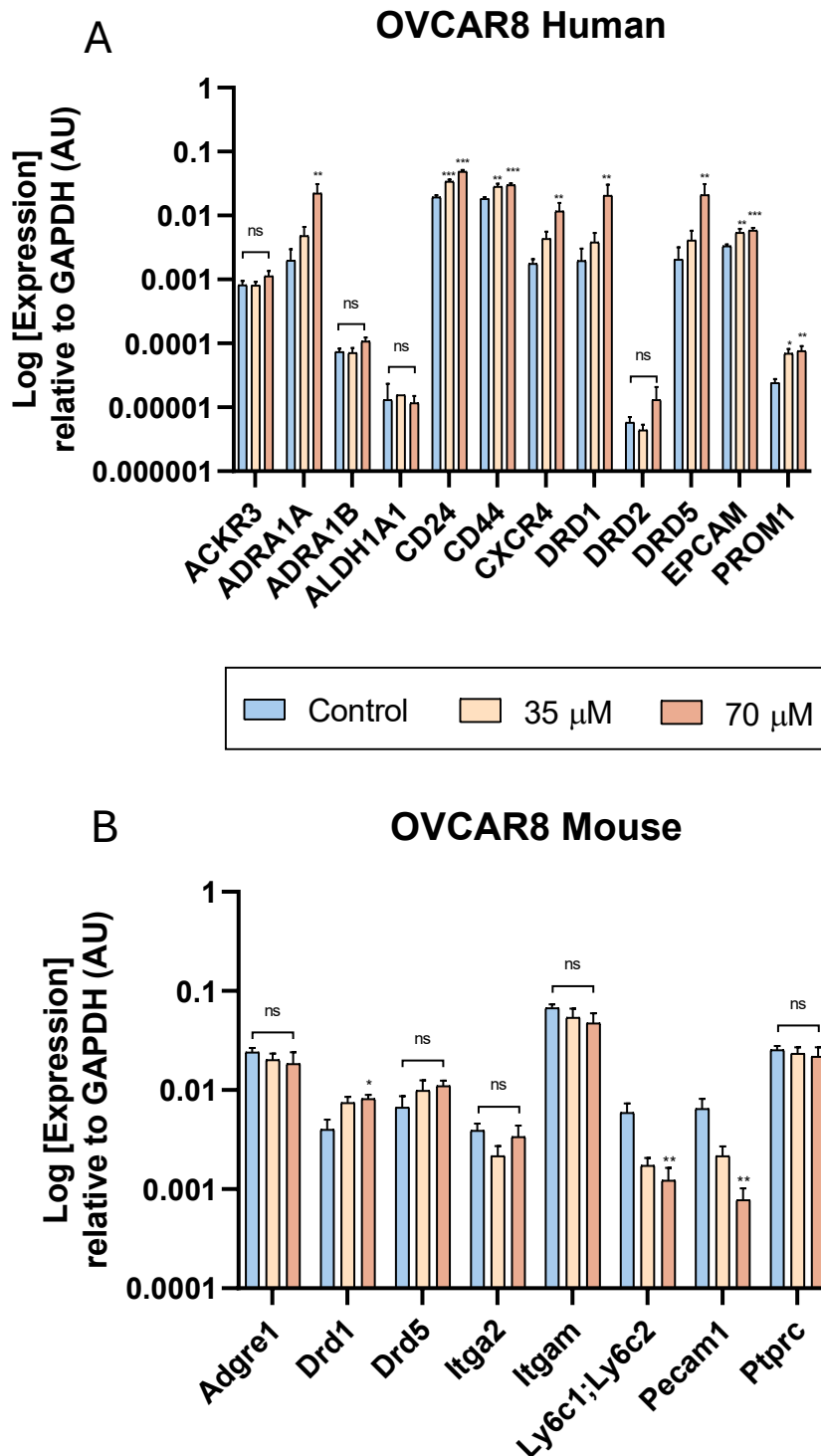


Figure 37. RT-qPCR analysis of human and mouse gene expression profiles in OVCAR8 tumour slices following 48h Domperidone treatment at 35 or 70 μ M. **(A)** Expression of human-specific genes relative to GAPDH. **(B)** Expression of mouse-specific stromal markers relative to GAPDH. Data are presented as $\log_{10}$ mean $\pm$ SEM. Statistical analysis was performed using Linear Mixed Models (LMM) and post hoc tests. $p < 0.05$, $**p < 0.01$, $***p < 0.0001$.

For ISHIKAWA, expression of three human genes were significantly altered upon treatment with 70 μ M Domperidone (Figure 38A). For instance, ADRA1B was upregulated by approximately 4-fold with a mean of 0.000757 compared to the Control (0.000191, $p = 0.0183$). Similarly, both the expression of CD44 and Kit were upregulated by two-fold compared to the Control group (Table 25). For ISHIKAWA mouse genes, many of the samples could not be quantified and utilised due to poor experimental duplicates during the amplification process and subsequently removed during data clean up. This resulted in a very small sample size ($n < 6$) with some conditions having only one or two data points, and consequently statistics could not be conducted, for instance for expression levels of DRD1, DRD5 and Adgre1 (Figure 38B). With the genes where statistics could be run, some main effects of the model were detected e.g., for Itga2 ($p = 0.000440$) and Pecam1 ($p = 3.22 \times 10^{-5}$), however statistically significant differences between the drug and control conditions were not detected, likely due to the small sample size.

Table 25. Summary of statistically significant changes in expression of human-specific genes ISHIKAWA slices treated with 35 and 70 μ M Domperidone for 48h.

Gene	Control	35 μ M	p-value (35 vs. ctrl)	70 μ M	p-value (70 vs. ctrl)
ADRA1B	0.000191	0.000254	0.9729	0.000757	0.0183
CD44	0.00572	0.01005	0.0818	0.01312	0.0222
EPCAM	0.0232	0.0263	ns	0.0400	0.0911 (ns)
KIT	1.07e-04	1.29e-04	0.9504	2.56e-04	0.0403

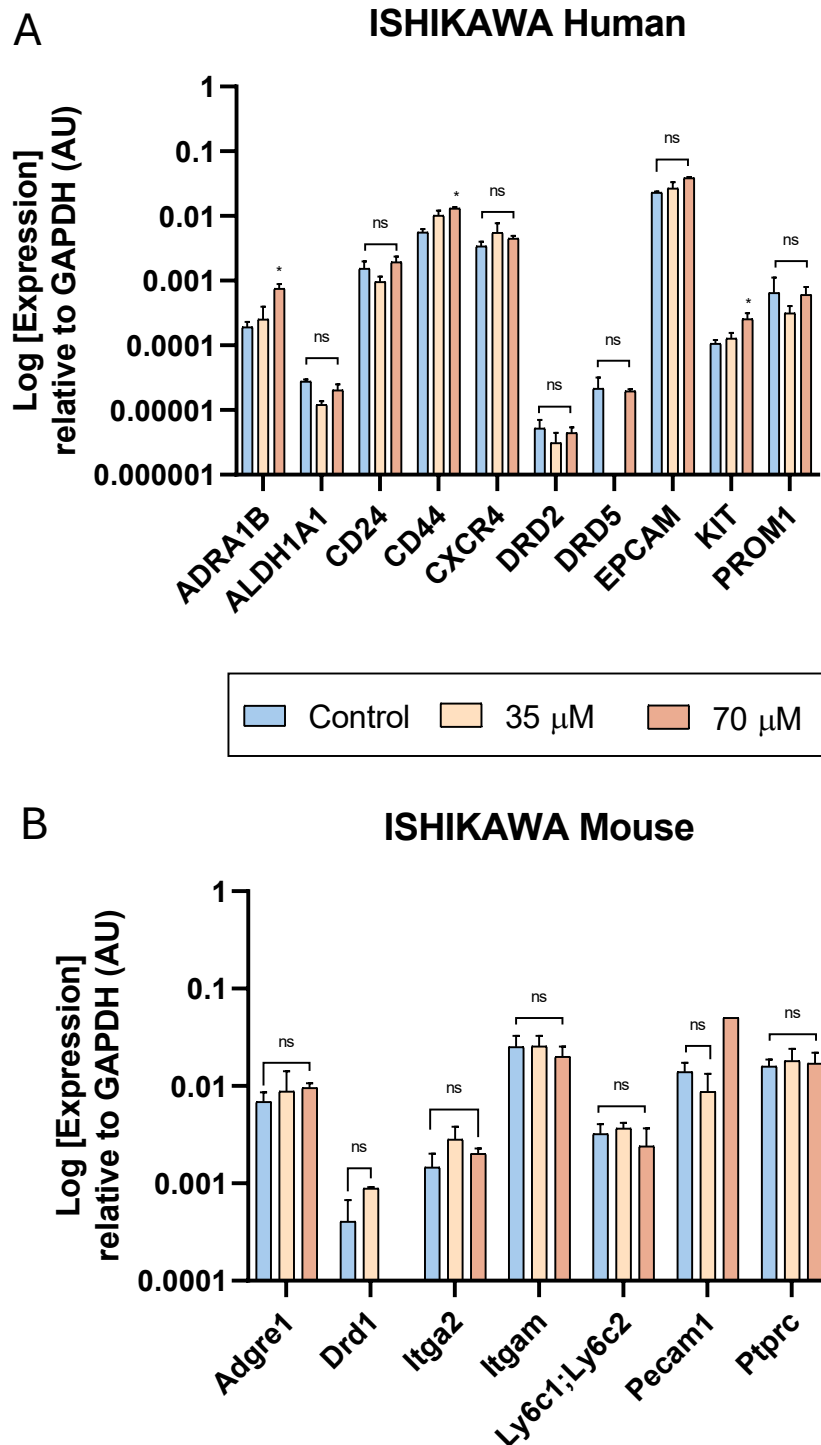


Figure 38. RT-qPCR analysis of human and mouse gene expression profiles in ISHIKAWA tumour slices following 48h Domperidone treatment at 35 or 70 μ M. (A) Expression of human-specific genes relative to GAPDH. (B) Expression of mouse-specific stromal markers relative to GAPDH. Data are presented as $\log_{10}$ mean $\pm$ SEM. Statistical analysis was performed using Linear Mixed Models and post hoc tests, $p < 0.05$.

4.2.7 Proteomic Analysis Reveals Potent MEK inhibition and DRD1 Compensation

To assess the downstream mechanisms of DRD2 blocking within OVCAR8 OTSCs, slices were treated with Domperidone (35 and 70 μ M) or media-only for 48h. Changes in protein expression of dopaminergic receptors and intracellular molecules involved in MEK/ERK (MAPK) and AKT/GSK-3 signalling pathways were immunoblotted and quantified.

Analysis of Western blots revealed distinct dose-dependent shifts in dopaminergic receptor levels and their downstream effector signalling molecules. OTSC slices treated with 35 and 70 μ M Domperidone experienced significant increases in DRD1 protein expression of 51% ($p = 0.0344$; ANOVA) and 84% ($p=0.0089$; ANOVA), compared to the control slices (Figure 39A). This could be visualised by increasing band intensities at ~50 kDa across the conditions in Figure 39B. Interestingly, dose-dependent, non-significant decreases in DRD2 protein levels of 26% ($p = 0.225$; ANOVA) and 44% ($p= 0.0744$; ANOVA) were also observed, along with decreasing band intensities at ~51 kDa (Figure 39B). Notably, β -arrestin-2 levels were also reduced by ~40% at 70 μ M, however this also did not reach statistical significance (Figure 39A).

When exploring effector molecules involved in the Raf/MEK/ERK (MAPK) pathway, an overall trend toward downregulation was observed, except in the last effector phosphorylated ERK (p-ERK). Slices treated with 35 and 70 μ M Domperidone demonstrated marked decreases in both total MEK levels (-37% and -69.1%, respectively) and phosphorylated-MEK levels (-50% and -75%, respectively) compared to Control slices. (Figure 39A). However, while ERK levels appeared to also decrease by approximately 25% across both concentrations of Domperidone, activated p-ERK exhibited varied responses, decreasing by 12% in the 35 μ M Domperidone, but increasing by 16.5% in the 70 μ M Domperidone treatment group. Due to high standard errors and small sample size ($n=2$) these differences often failed to reach statistical significance.

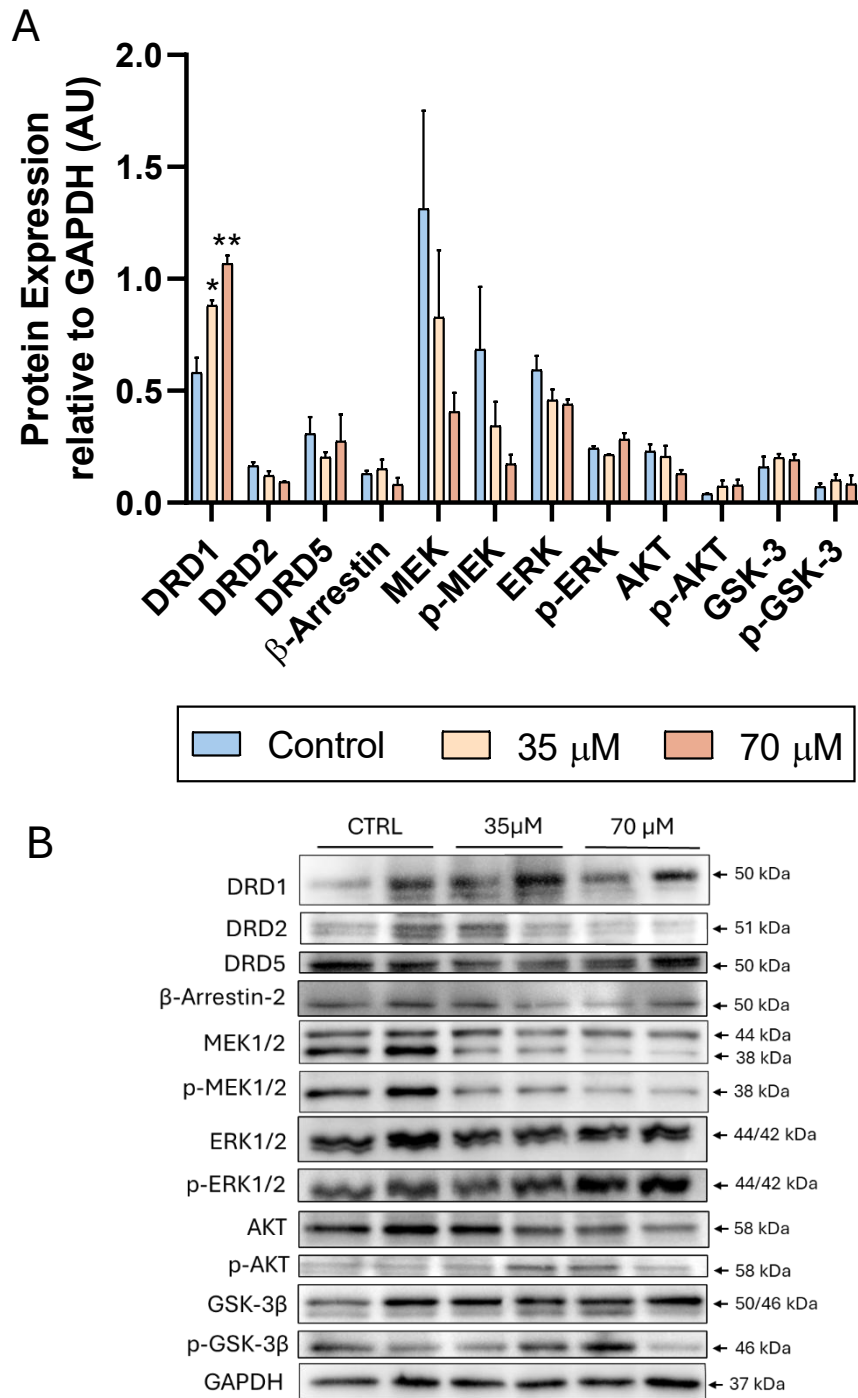


Figure 39. Effects of DRD2 antagonism (Domperidone) on protein expression in OVCAR8 OTSC slices. (A) Quantification of protein expression changes of DRD1, DRD2, DRD5, β -arrestin-2 and signalling molecules in the Raf/MEK/ERK (MAPK) and PI3K/AKT/GSK-3 signalling pathways in OVCAR8 OTSC treated with Domperidone (35 μ M and 70 μ M) or media-only for 48h. Proteins were immunoblotted and band intensity was quantified relative to GAPDH (AU). Plotted as mean ($n=2$) $\pm$ SEM with ANOVA statistical analysis, * $p<0.05$, ** $p<0.01$. **(B)** Representative Western blot images used for quantification of Figure A with estimation of molecular weight in kDa ($n=2$).

However, signalling molecules within the PI3K/AKT/GSK-3 signalling pathway exhibited activation. Total levels of AKT were reduced in a dose-dependent manner by ~10% and ~44% in the 35 μ M and 70 μ M Domperidone treatment groups, respectively (Figure 39A). Whereas p-AKT in its active phosphorylated state were upregulated with increasing Domperidone concentrations to values of 187.3% and 200.3%, compared to the control slices (100%). Interestingly, levels of AKT's downstream target, GSK-3 β , were found to be increased in both its unphosphorylated active state (associated with the negative regulation of β -catenin) by +20-27%, and its inhibited phosphorylated (Ser9) state (associated with β -catenin stabilisation) with varied responses of +42% and +16% for 35 μ M and 70 μ M treatment groups, respectively. This could be indicating that in response to downregulation of DRD2/ β -arrestin-2 and its downstream MEK/ERK and AKT signalling pathways, DRD1 could be being upregulated as a compensatory mechanism, maintaining pro-tumorigenic p-ERK and p-AKT/p-GSK-3 levels.

4.2.8 Patient Biopsy Pilot Study Validates Heterogeneous Responses to Domperidone

When assessing the viability of treated and non-treated ovarian cancer patient biopsy slices during a 3-day culture period, a linear mixed model revealed there was no significant differences between the two groups on Day 1 prior to treatment ($\chi^2(1) = 3.11$, $p = 0.078$), with post hoc analysis indicating both groups had an equal health baseline after 24 hours in culture ($t(4) = -1.76$, $p = 0.1528$). After 48h treatment with Domperidone, the treated group also did not statistically differ from the Control ($t(5) = 1.20$, $p = 0.2857$) (Figure 40A). When comparing a larger patient cohort using patient response data on Day 3 relative to individual metabolic baselines on Day 0, on average the control group had a viability of 62.2 % (SE ± 11.3) compared with the treatment group with 39.0% (SE ± 11.3). However, this 1.6-fold decrease in viability also did not reach statistical significance ($t(6) = -1.60$, $p = 0.1603$). Similarly, when data were normalised to the control slices (100%), Wilcoxon analysis of the median again revealed no statistical difference between the treated and control slices ($v = 7$, $p = 0.2719$) (Figure 40B). For cell death, LDH release was measured in the media of the ovarian cancer patient slices during 3-day culture, before and after treatment. Linear mixed modelling revealed that the control and treated slices

did not statistically differ 24h (Day 2, $t(2) = -0.414$, $p = 0.7188$) or 48h (Day 3, $t(2) = -0.470$, $p = 0.6845$) after treatment. Similarly, a one-way t-test of the control-normalised data also revealed a similar picture ($t(2) = 0.439$, $p = 0.703$). However, it should be noted that sample size was very small ($n = 3$) (Figure 40C-D).

Similarly, while the majority of the HGSOC patient samples (OV7, OV14 and OV22) appeared to respond strongly to Domperidone, with an average viability of over two-fold less (32.1%) compared to the matched Control group (67.8%), this also did not reach statistical significance ($t(2) = -1.14$, $p = 0.372$) (Figure 41A-B). However, it should be noted that a sample size of three is very small for this type of analysis. Interestingly, one of the highly responsive HGSOC samples (OV7) with a treatment-induced viability loss of 91% in the AlamarBlue analysis was also analysed using a LIVE/DEAD kit and confocal microscopy (Figure 41C). Confocal microscopy images revealed a huge loss of green-stained alive cells (83%) and a similar increase of red-stained dead cells in the Domperidone-treated slices. Similarly, one seemingly 'non-responsive' HGSOC sample (OV22) in the AlamarBlue metabolic assays (with a 33.1% increase in viability) demonstrated a large 170% increase in LDH release following a 48h treatment with 70 μM Domperidone, indicating cellular cytotoxicity (Table 26).

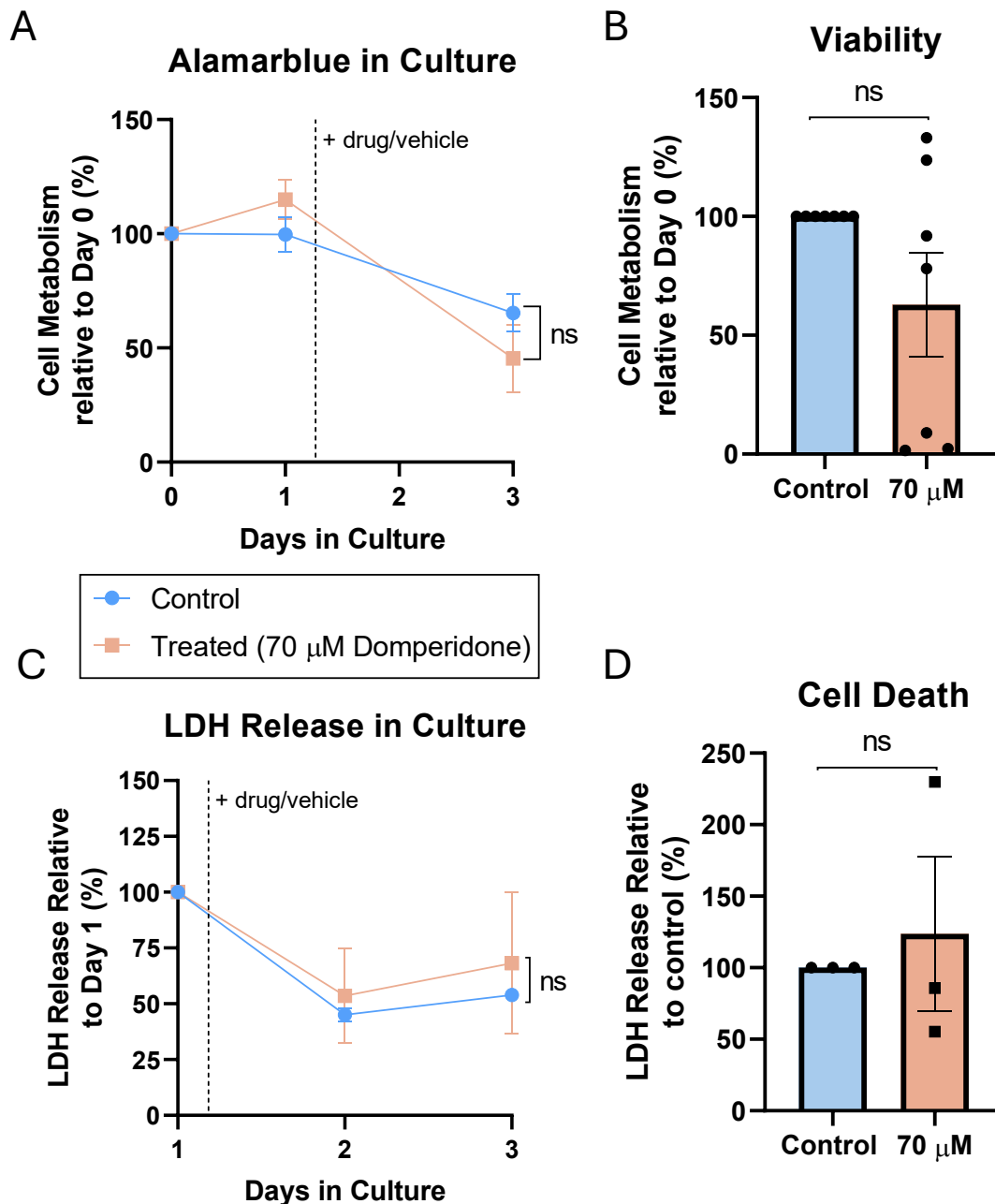


Figure 40. Effects of DRD2 antagonism (Domperidone) in ovarian cancer patient biopsy tumour slices. (A) Metabolic activity of patient tumour slices over a 3-day culture period, expressed relative to Day 0 ($n = 6$) treated with Domperidone (70 μ M) for 48h after a 24h recovery period in culture (dotted line). Viability was assessed using AlamarBlue and statistics by linear mixed modelling (LMM), ns = not significant. **(B)** Metabolic activity of treated slices on Day 3 expressed as a percentage of matched controls ($n = 7$) analysed by One-Sample t -test, ns. **(C)** Cell death measured by LDH release over a 3-day culture period ($n = 3$), ns (LMM). **(D)** Cytotoxicity (LDH release) of treated slices on Day 3 expressed as a percentage of control ($n = 3$); ns (One-Sample t -test. Data are presented as mean $\pm$ SEM.

Table 26. Summary of ovarian cancer patient samples, their confirmed diagnosis and OTSC response to 70 μ M Domperidone (Domp) for 48h with AlamarBlue, LDH release or confocal microscopy LIVE/DEAD analyses. Comparison of the xenograft OTSC models shown below.

Sample ID	Diagnosis	Response to 70 μ M Domp	Cell Viability %Change	Cell Death %Change
OV5	Endometrioid Carcinoma	Non-Responsive	+23.7	-5.5
OV7	HGSOC	Responsive	-91.0	83% *confocal
OV13	Borderline Mucinous	Non-Responsive	-8.2	n/a
OV14	HGSOC	Responsive	-98.4	n/a
OV19	Mucinous	Responsive	-97.7	+32.5
OV21	Borderline	Partial Response	-22.0	n/a
OV22	HGSOC	Partial Response	+33.1	+170
OVCAR8 Xenograft	HGSOC Model	Responsive	-36%	+63%
ISHIKAWA Xenograft	Endometrial Model (LG)	Partial Response	+36%	+128%

Note: Numerical figures in **bold** denote drug-responsiveness i.e. >20% reduction in cell viability or >20% increase in cell death. Responsive = >50% change in at least one metric (viability must be reduced); Partial Response = >20% change in at least one metric, not meeting Responsive criteria; Non-Responsive = <20% change across all metrics. Cell death measured by LDH release except *confocal (reduction in live cell fraction by calcein-AM/ethidium homodimer-1 staining). n/a = not measured.

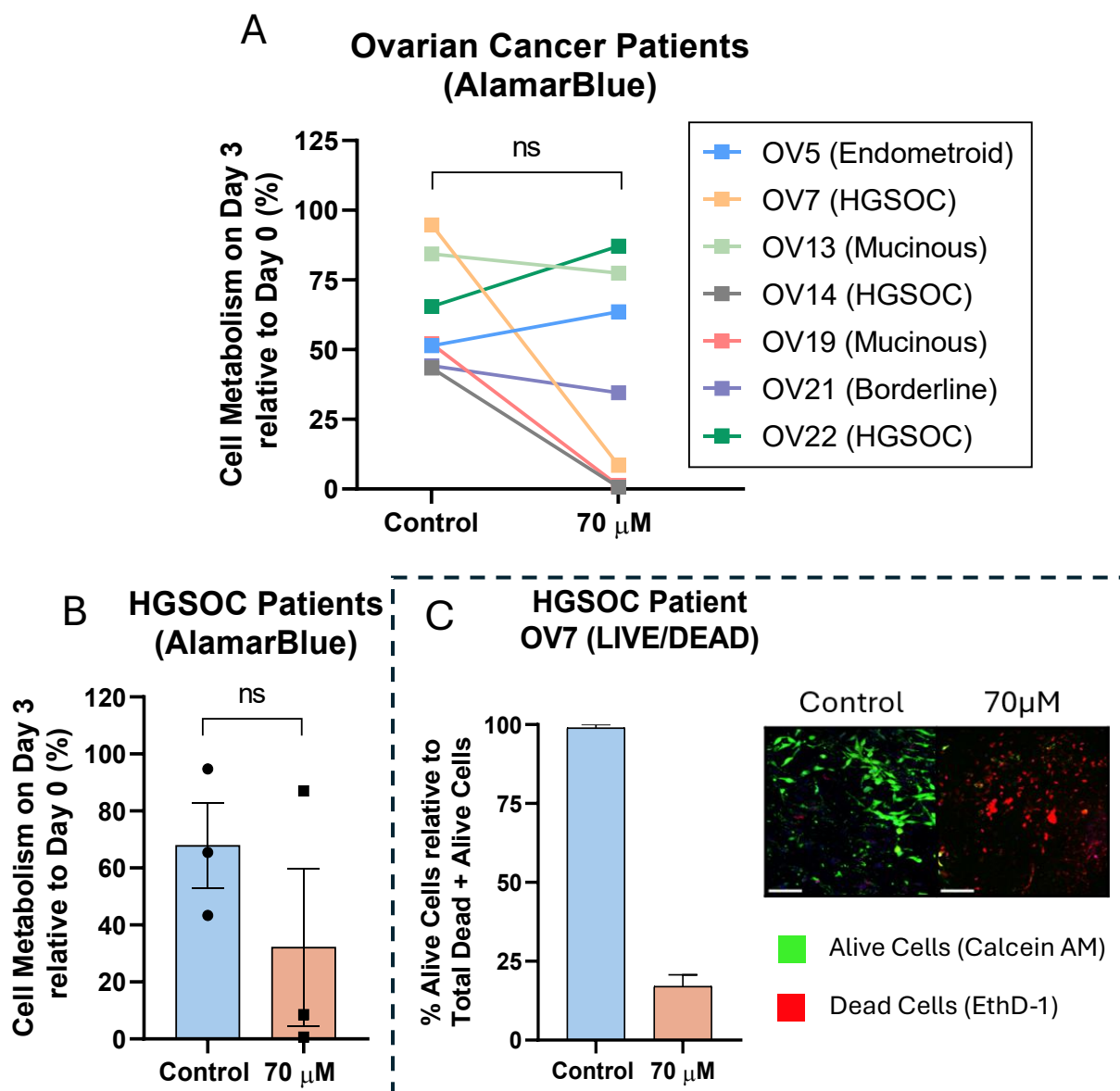


Figure 41. Effects of DRD2 antagonism (Domperidone) in HGSOC patient biopsy tumour slices. (A) Paired line graph of metabolic activity of ovarian cancer patient tumour slices treated with or without Domperidone (70 μ M) for 48h relative to Day 0 baseline measurements ($n=7$). Key demonstrates sample ID and confirmed clinical diagnosis. (B) Metabolic activity of HGSOC treated slices on Day 3 expressed as a percentage Day 0 baseline measurements ($n = 3$, ns. (C) Quantification and representative images of LIVE/DEAD confocal microscopy imaging of HGSOC patient (OV7) slices treated with or without Domperidone (70 μ M) and stained with calcein-am (green; alive cells), ethidium homodimer-1 (red, dead cells) and Hoechst (nucleus, blue), represented as mean of 2-3 slices per condition; scale bar = 50 μ m.

4.2.9 High DRD2 Predicts Opposing Survival Outcomes in Gynaecological Cancer

To assess the association between DRD2 expression and ovarian cancer, mRNA expression in ovarian cancer tissues ($n = 426$) from two primary datasets (TCGA and GTEx) was compared with normal ovarian samples ($n = 88$). As shown in Figure 42A, DRD2 expression was significantly upregulated in tumour tissue relative to normal controls. While normal samples displayed a relatively narrow distribution of low expression levels, tumour samples exhibited substantial heterogeneity.

To assess the clinical relevance of this finding, Kaplan–Meier survival analysis was performed over a 250-month follow-up period. Patients with high DRD2 expression showed a significantly improved overall survival compared to those with low expression. This was supported by a hazard ratio (HR) of 0.83 (95% CI: 0.72–0.95), indicating an approximately 17% reduction in mortality risk in the high-expression group. The difference between groups was statistically significant ($p = 0.0055$). The survival curves demonstrated a sustained separation over time, with a larger proportion of the high-expression group remaining alive at key intervals (e.g., 78 vs. 19 patients at 100 months; (Figure 42B). Collectively, these results indicate that although DRD2 is overexpressed in ovarian cancer relative to normal tissue, higher expression levels are paradoxically associated with improved patient survival, suggesting a potential role for DRD2 as a favourable prognostic biomarker.

Previously, we demonstrated that in endometrial cancer tissues there is also a significant association between DRD2 expression and patient outcome (Español et al. 2023). However, in contrast to ovarian cancer which demonstrates a positive correlation between DRD2 expression and survival, in endometrial cancer the opposite is found. As seen in Figure 42C, patients with low DRD2 expression (blue) exhibited significantly improved survival probabilities over time compared to those with high expression (red) ($p = 0.04$). The separation between the survival curves became more pronounced with increasing months, suggesting a sustained survival advantage in the low expression group. Correspondingly, the number-at-risk table showed a greater proportion of patients remaining in the low DRD2 group at successive time points (e.g., 50 patients vs. 6 at 50 months), further supporting the association between elevated DRD2 expression and worse overall survival.

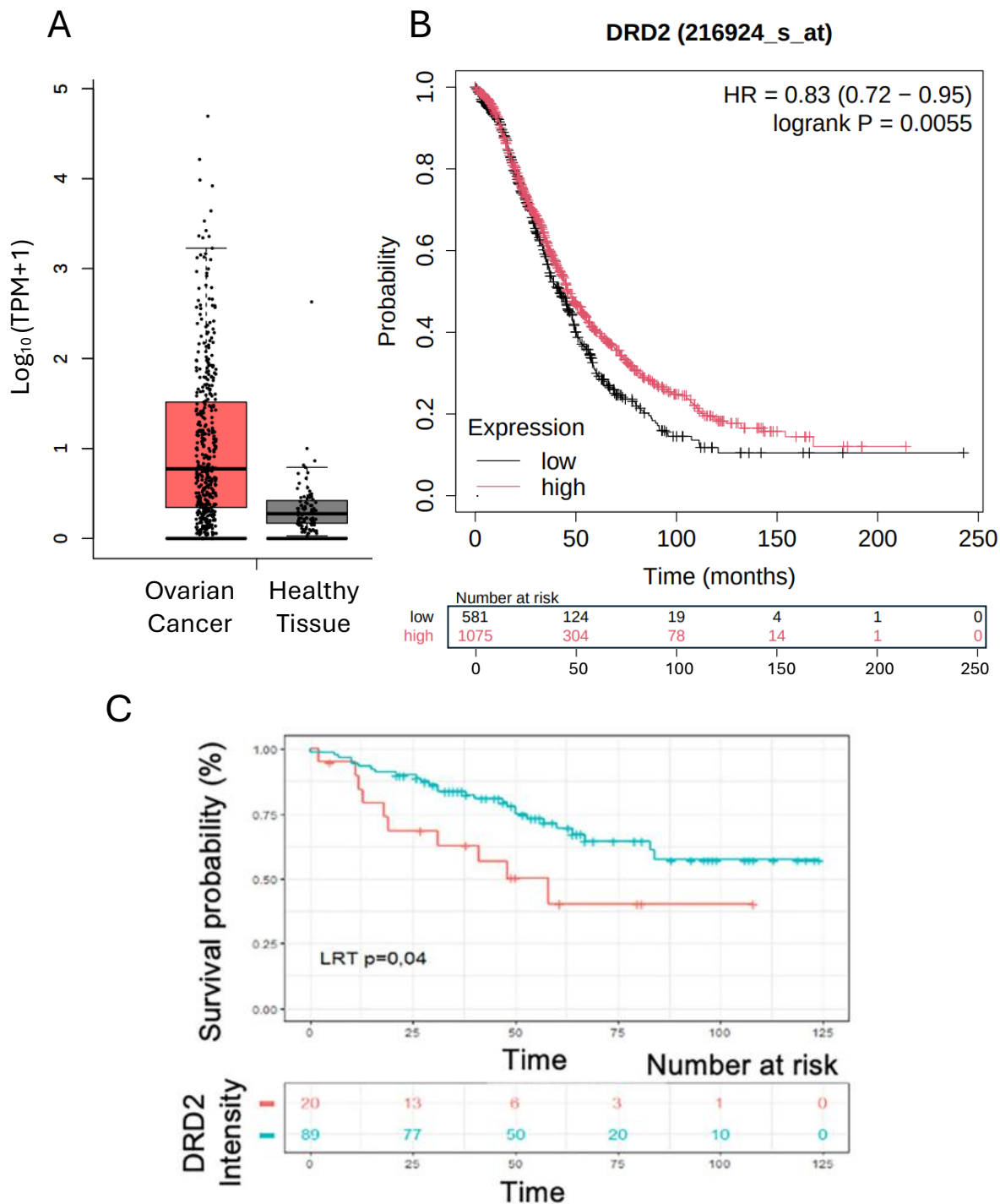


Figure 42. DRD2 Expression and Prognosis in Gynaecological Cancer . (A) Differential Expression of DRD2 in ovarian cancer tissues (n=426) compared to healthy controls (n=88). **(B)** Kaplan-Meier curves of overall survival for high DRD2 expression (red) and low DRD2 expression ovarian cancer cases. Number of patients remaining across 250 months for both expression cohorts shown below. **(C)** Data from Español et al. (2023) of overall survival Kaplan-Meier curves for high DRD2 (red) and low DRD2 (blue) IHC staining intensity in endometrial cancer. P value obtained using the Likelihood ratio test.

Chapter 5

The Development of Domperidone-Loaded Poly(Lactic-co-Glycolic Acid) Nanoparticles and Their Efficacy in Gynaecological Cell Lines and OTSC

While the findings in Chapter 4 established the potent anti-cancer and immunomodulatory effects of Domperidone within gynaecological OTSC models, its clinical translation faces significant pharmacological hurdles. Domperidone is an EMA-approved anti-emetic with a well-established safety profile at standard doses; however, its transition to oncology is limited by exceptionally low oral bioavailability (13–17%), low solubility and extensive plasma protein binding. Furthermore, Domperidone is associated with severe cardiotoxicity, including QT prolongation and arrhythmias.

To bridge this gap between *ex vivo* efficacy and clinical viability, it is necessary to optimise the drug's pharmacokinetic profile. Nanotechnology offers a transformative solution to these challenges. By encapsulating hydrophobic agents within polymeric nanoparticles, such as PLGA-PEG, we can enhance drug solubility, prevent premature metabolic degradation, and minimise systemic off-target effects. Moreover, these nanocarriers leverage the enhanced permeability and retention (EPR) effect, facilitating the targeted accumulation of the therapeutic payload directly within the tumour site.

Chapter Aims

The primary objective of this chapter is to engineer and validate a nanoparticle-based delivery system for Domperidone. The specific aims are to:

- **Develop and Characterise the Nanocarrier:** Establish a protocol for the synthesis of Domperidone-encapsulated PLGA-PEG nanoparticles, evaluating their size and drug-loading efficiency.
- **Evaluate Cellular Internalisation:** Investigate the uptake and cytotoxicity of the nanodrug within 2D gynaecological cancer monolayers.
- **Validate 3D Efficacy:** Explore the therapeutic performance and biodistribution of the nano-encapsulated drug within the validated OTSC platform.

The findings from this chapter will establish a novel delivery strategy to improve the bioavailability and therapeutic window of Domperidone for the treatment of ovarian and endometrial cancers.

5.1 Methods

5.1.1 Nanoparticle Synthesis

Domperidone-loaded nanoparticles were prepared using an amphiphilic block copolymer of poly(ethylene glycol)-block-poly(lactic-co-glycolic acid) (PEG–PLGA) via the oil-in-water nanoprecipitation method. First, Domperidone was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL. The organic phase was prepared by dissolving 25 mg PEG–PLGA (50:50, Sigma, #900948) in 2.4 mL acetone with or without 0.1 mg rhodamine b (using 100 μ L of a 1 mg/mL stock solution) and subsequently adding 0.1 mL of the Domperidone/DMSO solution (1 mg drug, $\leq 10\%$ DMSO v/v) or 0.1 mL DMSO for control nanoparticles (Table 27). The mixture was vortexed and sonicated until fully solubilised. The aqueous phase was prepared with 12.5 mL 2% Polyvinyl Alcohol (Sigma) under magnetic stirring (800 rpm). The organic phase was then added dropwise into the aqueous phase using a syringe, resulting in the spontaneous self-assembly PLGA-PEG nanoparticles containing hydrophobic Domperidone within the hydrophobic core of the copolymer. The suspension was stirred overnight at room temperature in a hood to allow solvent evaporation (Figure 43).

To remove unencapsulated drug, the suspension was centrifuged at 12,000 $\times g$ for 5 min, and the pellet containing the nanoparticles was collected and resuspended in 1 mL Milli-Q water. The suspension was centrifuged and washed twice more to remove any excess PVA and/or rhodamine B (Figure 43). The initial supernatant from the first wash containing free Domperidone was filtered (0.22 μ m; Millipore Millex PES Syringe Filter) and analysed by UV–Vis spectrophotometry at 284 nm. A Domperidone calibration curve (15.6–200 μ g/mL in DMSO) was used to quantify the concentration of free drug (C_{free}) in the supernatant. This value, alongside the volume of the supernatant (V_{free}), were used to calculate the mass of free (M_{free}) Domperidone:

Mass of unencapsulated Domperidone (M_{free}):

$$M_{free} = C_{free} \times V_{free} \quad (9)$$

By subtracting the mass of the free unencapsulated drug calculated above from the starting mass of Domperidone (M_{Initial}), the mass of encapsulated Domperidone ($M_{\text{encapsulated}}$) could be deduced:

Mass of encapsulated Domperidone ($M_{\text{encapsulated}}$):

$$M_{\text{encapsulated}} = M_{\text{Initial}} - M_{\text{free}} \quad (10)$$

Encapsulation efficiency (EE%) could then be calculated by dividing the mass of the encapsulated Domperidone by the starting mass used in the experiment:

Encapsulation Efficiency:

$$\text{Encapsulation Efficiency (\%)} = \frac{M_{\text{Encapsulated}}}{M_{\text{Initial}}} \times 100 \quad (11)$$

Finally, drug loading (DL%) was then estimated by dividing the mass of the encapsulated Domperidone by the total mass of encapsulated Domperidone added to the mass of the polymer (PLGA-PEG) used in the reaction:

Drug Loading:

$$\text{Drug Loading (\%)} = \frac{M_{\text{Encapsulated}}}{M_{\text{Encapsulated}} + M_{\text{polymer}}} \times 100 \quad (12)$$

Table 27. Names of the different PEG-PLGA formulations loaded with or without Domperidone or Rhodamine B and the masses of reagents used.

Name	PEG-PLGA	Domperidone	Rhodamine
Domp-PLGA-PEG	25 mg	1 mg	n/a
Control- PLGA-PEG	25 mg	n/a	n/a
Domp-PLGA-PEG -Rhod	25 mg	1mg	0.1 mg
Control-PLGA-PEG-Rhod	25 mg	n/a	0.1 mg

To improve the homogeneity of the nanoparticles and remove large-sized or aggregated particles, the Domperidone-loaded PEG–PLGA nanoparticles (Domp-PLGA-PEG NPs) were filtered using a 0.8 µm MCE membrane syringe filter (Millipore Millex®).

5.1.2 Nanoparticle Characterisation

The hydrodynamic diameter and polydispersity index (PDI) of the Domp-PLGA-PEG NPs were measured by dynamic light scattering (DLS) on the Zetasizer Lab and ZS Xplorer analytical software (Malvern Panalytical) (Figure 43). Samples were diluted in deionised water to an appropriate concentration and analysed at 25 °C in disposable cuvettes. Measurements were performed in triplicate, and data were reported as the mean ± standard deviation.

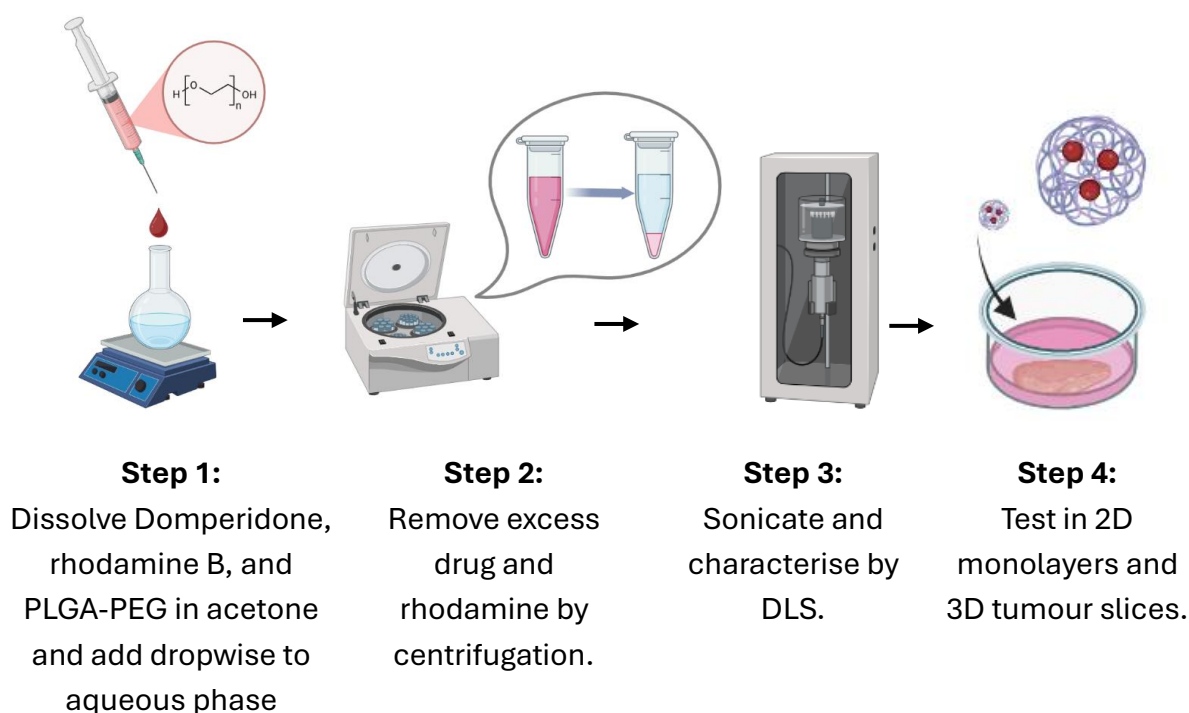


Figure 43. Diagram demonstrating the protocol for the development of Domperidone and Rhodamine B-loaded PLGA-PEG nanoparticles (NPs) using the oil-in-water method. The NPs were washed by centrifugation, sonicated and characterised by Dynamic Light Scattering (DLS). The NPs were then tested in the cell lines grown in 2D monolayers of 3D ex vivo OTSC slices of OVCAR8 and ISHIKAWA cells.

5.1.3 Quantification of Domperidone in PLGA-PEG NPs by LC-MS/MS

The concentration of domperidone encapsulated within PLGA-PEG nanoparticles was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS), performed by the Applied Metabolomics Research Group (MarMetabolomics Platform, Hospital del Mar Research Institute, IMIM, Barcelona). Briefly, nanoparticle samples (20 μ L) were treated with 0.2 M NaOH for 30 min at 37°C with gentle mixing to disrupt the polymeric matrix and release the encapsulated drug, followed by centrifugation at 14,500 rpm for 5 minutes. Final extracts were prepared by mixing 10 μ L of the treated nanoparticle solution with 10 μ L of internal standard (cholic acid-d₄, 5 μ g/mL) and 80 μ L of methanol/water (1:1), and 2 μ L were injected into the LC-MS/MS system (Acquity I-Class UPLC coupled to Xevo TQ-S Micro triple quadrupole, Waters). Chromatographic separation was performed on an Acquity BEH C18 column (Waters) at 55°C, with a mobile phase of water and methanol both containing 1 mM ammonium formate and 0.01% formic acid. Data were acquired in positive ionisation mode using multiple reaction monitoring (MRM). Quantification was achieved against a calibration curve (15.6–250 μ g/mL) using the ratio of domperidone to internal standard peak areas. MassLynx V4.2 and TargetLynx XS (Waters) software were used for data acquisition and processing.

5.1.4 2D Cell Line Viability Assays with Domp-PLGA-PEG NPs

For 2D monolayer cell line viability studies, cell lines were seeded in 96-well plates at a density of 1×10^5 cells/mL in 0.1 mL of appropriate media, grown for 24h (~70% confluency) and treated with Domp-PLGA-PEG NPs or vehicle-only for 48h in standard cell culture conditions. Domp-PLGA-PEG NPs were used at concentrations of 0, 25, 50, 100, 200, and 500 μ M, estimated from mass of encapsulated Domperidone ($M_{\text{Encapsulated}}$) described above in Equations 9-10. Control NPs were added at the same dilutions used for the Domperidone-loaded NPs. Viability was measured using the 1X AlamarBlue viability dye as described before in Chapter 3.

5.1.5 OTSC Viability Assays with Domp-PLGA-PEG NPs

ISHIKAWA and OVCAR8 tumours cells were implanted in mice, resected, sliced and cultured according to our developed methodology in Chapter 3. After 24h of slice recovery, the media was replaced with fresh media containing either Control NPs or filtered Domp-PLGA-PEG NPs at a 1:10 dilution ($\sim 8 \mu\text{M}$ as determined by mass spectrometry) and incubated for 48h. Real-time AlamarBlue and LDH assays were employed, as before, to assess viability and cytotoxicity after treatment (see Chapter 3). Statistical analysis was conducted with LMM.

5.1.6 Study of Domp-PLGA-PEG NPs Internalisation and Biodistribution

To study whether the Domp-PLGA-PEG NPs are successfully internalised in the cells and to determine at which time point internalisation takes place, the nanoparticles were administered to the 2D monolayers and 3D OTSC slices and internalisation visualised by confocal microscopy after 1 and 2h. The ISHIKAWA and OVCAR8 cell lines were seeded at 2×10^5 cells/mL in 35mm glass-bottom dishes. To ensure optimal adherence and growth, the dishes were pre-treated with poly-D-lysine for 30 min, rinsed twice with sterile water and seeded with cells in 0.5 mL media. The cells were left 24h to reach 70% confluency in standard culture conditions. For OVCAR8 OTSC slices, the slices were prepared as per Chapter 3 and left to recover in appropriate media for 24h, rocking at 70 RPM in standard culture conditions. The following day, 50 μL rhodamine B-loaded Domp-PLGA-PEG were added to the dishes (final dilution 1:10; $\sim 8 \mu\text{M}$) and returned to the incubator. At the 1h and 2h time-points, the dishes were washed in PBS and cells/slices stained for 1 min with the cell membrane stain CellMask™ Deep Green (4 $\mu\text{g/mL}$) and nuclear stain Hoechst 33342 (5 $\mu\text{g/mL}$) diluted in phenol red-free media. Dishes consisting of cells treated with media-only were stained with either CellMask™ only or free rhodamine at 0.1 mg/mL and used as single stain controls. Then, the media was removed and cells washed twice with 1mL PBS and imaged immediately under the confocal microscope at 63X magnification (Leica).

5.2 Results

5.2.1 Variable Size and Concentration of Domp-PLGA-PEG NPs

The Domperidone-loaded PLGA-PEG NPs were prepared using the oil-in-water method and the size was measured using Dynamic Light Scattering (DLS). To estimate the concentration of Domperidone loaded inside the polymeric nanoparticles, the supernatant was collected from pelleting and washing the NPs and absorbance was measured using a spectrometer. The concentration of free Domperidone was calculated by interpolation of a calibration curve of known Domperidone concentrations, and this was then used to calculate the concentration of Domperidone loaded within the NPs. Control PLGA-PEG NPs were prepared in the same manner without Domperidone.

The resulting NPs varied in average size between 498.9-1190 nm and were composed of heterogeneously sized populations of particles (Table 28; Figure 44A). These populations typically formed two predominant peaks in DLS analysis, with one population consisting of smaller particles (143.1 – 769.9 nm) and the other much larger (777.7 – 4431 nm). Some batches, for example #3 and #5, had approximately 50% of the smaller population and 50% of the larger population. Whereas batch #1 was predominantly (90.4%) composed of the larger population with an average size 2109 nm (Table 28). In contrast, the calculated concentration determined by absorbance remained consistent ranging from 427.1 to 604.5 µg/mL. Later, a more accurate concentration of Domperidone was calculated from mass spectrometry and interpolation of a Domperidone standard curve. Results show that spectrometry-based calculations overestimated the concentration of Domperidone loaded in the nanoparticles by approximately 5-fold, with mass spectrometry concentrations ranging from 76.1 to 189.9 µg/mL (Table 28).

The control nanoparticles had an average size of 280.4 nm and were also composed of two populations. The most abundant (89.4%) was the smaller population with an average size of 310 nm, whereas the other 10% consisted of much larger particles (4583 nm). Spectrometry measurements confirmed the absence of Domperidone within the control nanoparticles (Table 28).

Table 28. The average size and size percentage peaks of unfiltered Domperidone or Control PLGA-PEG NPs. Concentrations calculated from Domperidone standard curves using spectrometry (absorbance at 284nm; abs) or mass spectrometry (MS).

ID	Average Size (nm)	PI	Peak 1 nm (% area)	Peak 2 nm (% area)	Conc. (µg/mL) Abs	Conc. (µg/mL) MS
1	1190	0.534	250.1 (8.2%)	2109 (90.4%)	439.4	76.1
2	653.6	0.985	769.9 (50.9%)	4431 (31.6%)	449.2	95.8
3	518.9	0.838	429.1 (41.1%)	2721 (51.6%)	506.1	170.2
4	550.4	0.466	143.1 (23.0%)	777.7 (77.0%)	427.1	130.8
5	498.9	0.781	197.9 (51.5%)	832.2 (47.1%)	604.5	189.9
CTRL	280.4	0.4025	310.5 (89.4%)	4583 (10.6%)	-	<0.2

5.2.2 Optimised Filtration Protocols Enhance Nanocarrier Homogeneity

In order to isolate the smaller population of nanoparticles, the NP suspensions were filtered using a 0.8 µm filter unit. The resulting batches had a much cleaner size profile, with many of the batches displaying a singular peak at ~160-178.6 nm (Table 29). While three batches (#11-13) displayed a secondary peak, these consisted of small sized particles ranging from 10-80 nm. In contrast, one batch (#10) exhibited either poor filtration or clumping following filtration with 11.6% of the suspension containing particles over 5000 nm and was subsequently removed from analysis and testing. As spectrometry-based calculations utilise free Domperidone in the supernatant to estimate the concentration loaded into the NPs, it was not possible to use this method to obtain an estimate of the concentration within the remaining nanoparticles following

filtration. However, later calculations using mass spectrometry demonstrated that the filtered NPs contained on average approximately 4-fold lower amount of Domperidone than the unfiltered NPs (34.2 vs 132.6 $\mu\text{g/mL}$; Figure 44B), with two batches consisting of relatively high concentrations of 59.0 and 78.0 $\mu\text{g/mL}$, one batch with approximately half of this concentration (28.0 $\mu\text{g/mL}$), and two batches with relatively low concentrations (2-4 $\mu\text{g/mL}$) (Table 29). This reduction in total Domperidone is to be expected due to earlier calculations estimating only ~8-50% of the particles were below the filtration cut off of 0.8 μm (Table 28). Three batches were measured by DLS but were not quantified by MS. Control NPs had no detectable levels of Domperidone (<0.2 $\mu\text{g/mL}$) and were predominantly composed (96.2%) of small ~310 nm sized particles, but also displayed some poor filtration or clumping with 3.7% of particles measuring 4897 nm post-filtration.

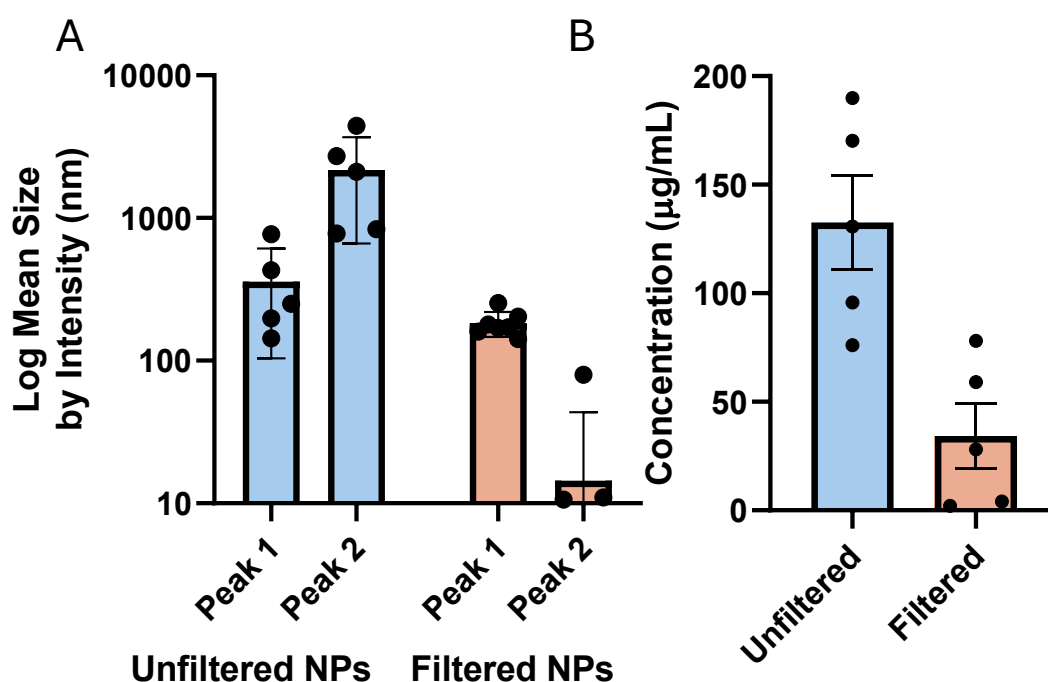


Figure 44. Size and Concentration of Domp-PLGA-PEG NPs. (A) The size of unfiltered and filtered Domperidone-loaded PLGA-PEG nanoparticles (NPs) determined by intensity via DLS. Data presented as $\log_{10}$ mean size (nm) $\pm$ SEM in peaks 1 and 2 (if present). (B) Concentration of unfiltered and filtered NPs determined by mass spectrometry and interpolation of a Domperidone standard curve. Presented as mean concentration $\pm$ SEM.

Table 29. The average size and size percentage peaks of 0.8 μm filtered Domperidone or Control PLGA-PEG NPs. Concentrations calculated from Domperidone standard curves using mass spectrometry.

ID	Date	Size (nm)	PI	Peak 1 nm (% area)	Peak 2 nm (% area)	Conc. ($\mu\text{g/mL}$)
6	13-08-25 A	149.2	0.157	178.6 (100%)	-	78.0
7	13-08-25 B	135.4	0.216	171.1 (100%)	-	28.0
8	19-8-25	138.7	0.180	169.6 (100%)	-	59.0
9	26-08-25	132.5	0.166	160 (100%)	-	2.0
10	17-09-25	350.5	0.406	269.3 (77.3%)	5120 (11.6%)	4
11	01-10-25	525.5	0.515	253 (49.2%)	79.48 (50.8%)	n/a
12	05-11-25	46.73	0.933	141.4 (77.5%)	11.69 (18.0%)	n/a
13	06-11-25	63.69	0.572	204 (86.6%)	10.57 (13.3%)	n/a
CTRL	26-08-25	233.9	0.373	310.9 (96.2%)	4897 (3.74%)	<0.2

5.2.3 Domp-PLGA-PEG NPs Reduce Viability of ISHIKAWA Cell Line

To test the cytotoxic effect of the Domp-PLGA-PEG NPs, the filtered nanomedicine was diluted to 25-500 μM using the concentration calculated from spectrometry absorbance readings (prior to filtering) and incubated for 48h with OVCAR8 and ISHIKAWA cell lines seeded in a 2D format in 24-well plates.

For the OVCAR8 cell line, Domperidone-loaded PLGA nanoparticles did not appear to cause a significant change in viability (Figure 45A). While the highest concentration of 500 μM achieved a viability of 87.3% compared to the media-only control, this was not significantly different from the control nanoparticles at the same concentration (mean = 94.45%). Conversely, at 50 μM , viability of the OVCAR8 cells increased to 104.7% and this was significantly greater than the Control PLGA-PEG NPs (95.65%, $p = 0.015$).

For the ISHIKAWA cell line, a decline in viability was observed with increasing concentrations of the Domp-PLGA-PEG NPs, reaching a significant reduction at 200 μM concentration compared with the control PLGA-PEG NPs (Wilcoxon Rank Sum Test, $p = 0.028$) (Figure 45B). Lower concentrations (25-100 μM) did not exhibit significant cytotoxic effects. Similarly, the highest concentration at 500 μM also did not significantly differ from the control NPs, nor did it cause further decline in viability compared with the Domperidone NPs.

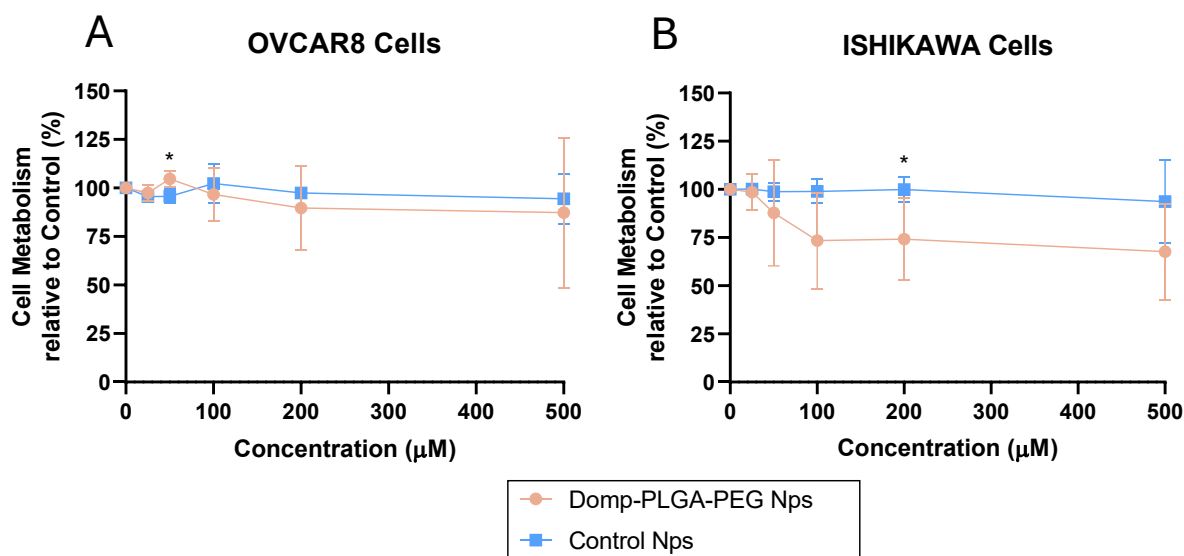


Figure 45. Viability of OVCAR8 and ISHIKAWA cell lines treated with Domp-PLGA-PEG NPs. Cell metabolism of **(A)** OVCAR8 and **(B)** ISHIKAWA cell lines seeded at 1×10^5 cells/well in 96 well plates and treated with 25-500 μM Domperidone-loaded PLGA-PEG Nanoparticles (NPs) for 48h or control (unloaded) NPs diluted at the same concentration. Presented as mean viability relative to the control wells (media-only) $\pm$ SEM. Statistical analysis conducted by t-test or Wilcoxon rank-sum test between Domperidone-loaded NPs and control NPs conditions, * $p < 0.05$.

5.2.4 Negligible Efficacy of Domp-PLGA-PEG NPs in OVCAR8 OTSC

We then wanted to test the Domp-PLGA-PEG NPs in our OTSC slices and explore the effects of viability and cell death within the 3D system heterogenic system. The slices were prepared as before, and following a 24h recovery period, the filtered NPs were incubated at a 1:10 dilution ($\sim 8 \mu\text{M}$) for 48h with OVCAR8 and ISHIKAWA 3D slices for 48h.

OVCAR8 slices did not display any significant changes upon treatment with the nanoparticles. In fact, mean viability on Day 3 for treated OVCAR8 slices was 14.8% higher than the control NP-treated slices, however this did not reach statistical significance in a linear mixed model analysis ($p = 0.335$), nor did a one-sample T-test of the normalised data ($p = 0.804$) (Figure 46A-B). Similarly, LDH release did not significantly differ in the Domp-PLGA-PEG NP treated group compared to the Control NP group on Day 3 ($p = 0.199$), with Control-normalised data displaying a small insignificant increase of 4.7% in LDH ($p = 0.623$) (Figure 46C-D).

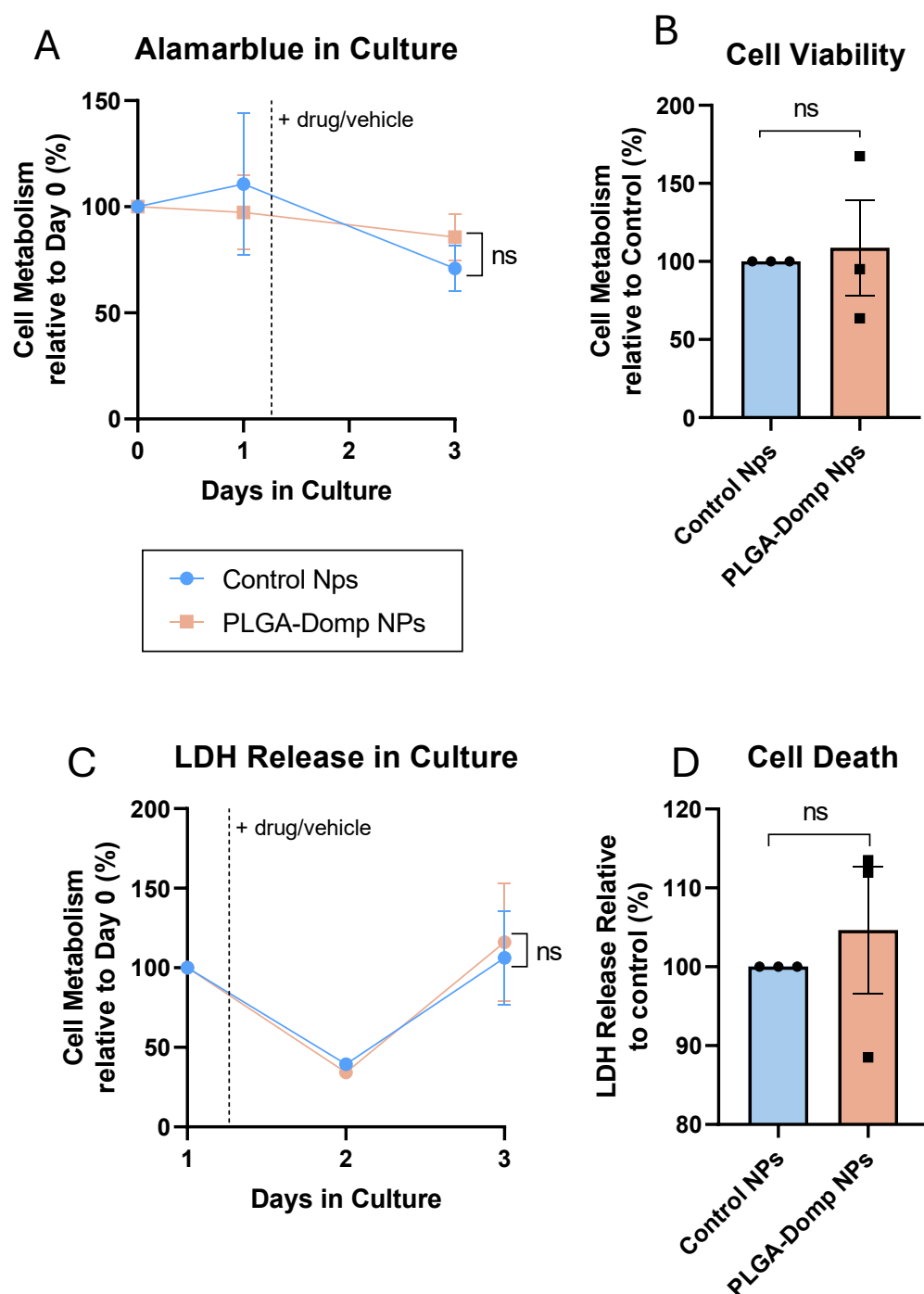


Figure 46. Effects of Domp-PLGA-PEG NPs in OVCAR8 tumour slices. (A) Metabolic activity of OVCAR8 tumour slices treated with control or Domperidone-loaded PLGA-PEG NPs at a 1:10 dilution ($\sim 8 \mu\text{M}$) for 48h after a 24h recovery period in culture (dotted line), expressed relative to Day 0 ($n = 3$). Viability was assessed using AlamarBlue and statistics by linear mixed modelling (LMM), ns = not significant. (B) Metabolic activity of treated slices on Day 3 expressed as a percentage of matched controls ($n = 3$) analysed by One-Sample *t*-test, ns. (C) Cell death measured by LDH release over a 3-day culture period ($n = 3$), ns (LMM). (D) Cytotoxicity (LDH release) of treated slices on Day 3 expressed as a percentage of control ($n = 3$); ns (One-Sample *t*-test). Data are presented as mean $\pm$ SEM.

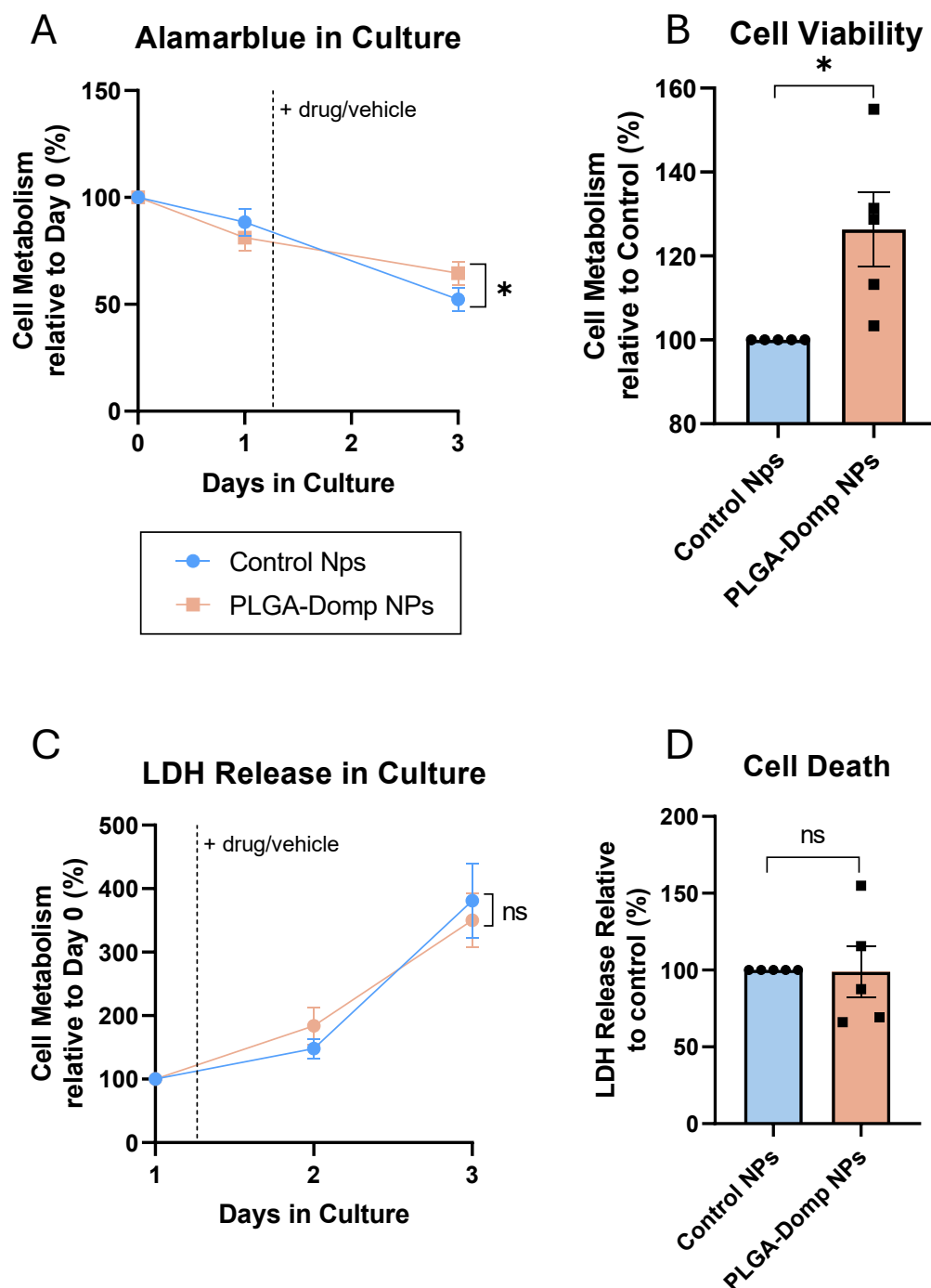


Figure 47. Effects of Domp-PLGA-PEG NPs in ISHIKAWA tumour slices. (A) Metabolic activity of ISHIKAWA tumour slices treated with control or Domperidone-loaded PLGA-PEG NPs at 1:10 dilution ($\sim 8 \mu\text{M}$) for 48h after a 24h recovery period in culture (dotted line), expressed relative to Day 0 ($n = 3$). Viability was assessed using AlamarBlue and statistics by linear mixed modelling (LMM), ns = not significant. **(B)** Metabolic activity of treated slices on Day 3 expressed as a percentage of matched controls ($n = 3$) analysed by One-Sample *t*-test, ns. **(C)** Cell death measured by LDH release over a 3-day culture period ($n = 3$), ns (LMM). **(D)** Cytotoxicity (LDH release) of treated slices on Day 3 expressed as a percentage of control ($n = 3$); ns (One-Sample *t*-test). Data are presented as mean $\pm$ SEM. * $p < 0.05$.

5.2.5 Domp-PLGA-PEG NPs Paradoxically Increase ISHIKAWA Slice Viability

Similar to OVCAR8 slice results, ISHIKAWA slices also displayed an increase in cell viability following 48-treatment with Domp-PLGA-PEG NPs compared with the Control NPs. This 12.3% increase reached statistical significance in linear mixed model analysis ($p = 0.0238$), and in the T-test with the Control-normalised data ($p = 0.0402$). However, both control and treated slices experienced large loss of viability (~40-50%) after 3 days in culture (Figure 47A-B). Likewise, both conditions experienced comparable increases in LDH release from their baseline measurements with values of 350.3% and 381% for Domperidone-loaded and Control NPs, respectively, ($p = 0.606$). This was confirmed by the Control-normalised data demonstrating 98.7% viability of the Control NPs ($p = 0.941$) (Figure 47C-D).

5.2.6 Confocal Tracking Confirms Time-Dependent Intracellular NP Accumulation

To explore internalisation of rhodamine Domp-PLGA-PEG NPs, ISHIKAWA and OVCAR8 cell monolayers were treated with NPs and analysed via confocal microscopy. Cell membranes were counterstained with CellMask™ (green) and nuclei with Hoechst (blue) to track the red-fluorescent NPs over time (Figure 48).

In 2D OVCAR8 monolayers, confocal imaging revealed distinct time-dependent internalisation profiles. As seen in Figure 48, at 1-hour incubation, NPs primarily localised at the cell membrane, with split-channel images demonstrating a clear red and green ring surrounding the cell nuclei (blue) resulting in a yellow overlap in composite images, that indicated a direct association of the NPs with the cell membrane. In contrast, at 2-hour incubation, enhanced intracellular accumulation was observed, with nanoparticles successfully internalised into the cytoplasm and distributed around the nuclei. This was evidenced by composite images showing a diminishing yellow fluorescence at the cell membrane, and with images now displaying small red puncta intracellularly within green-stained cell membranes. At 4h incubation, the intracellular NPs remained, however the fluorescence appeared slightly diminished in intensity and pixel size, which could indicate either NP degradation or NP extracellular trafficking. To confirm specificity of the red fluorescence, media-only controls stained with CellMask™, showed a total absence of red fluorescence (Figure 48).

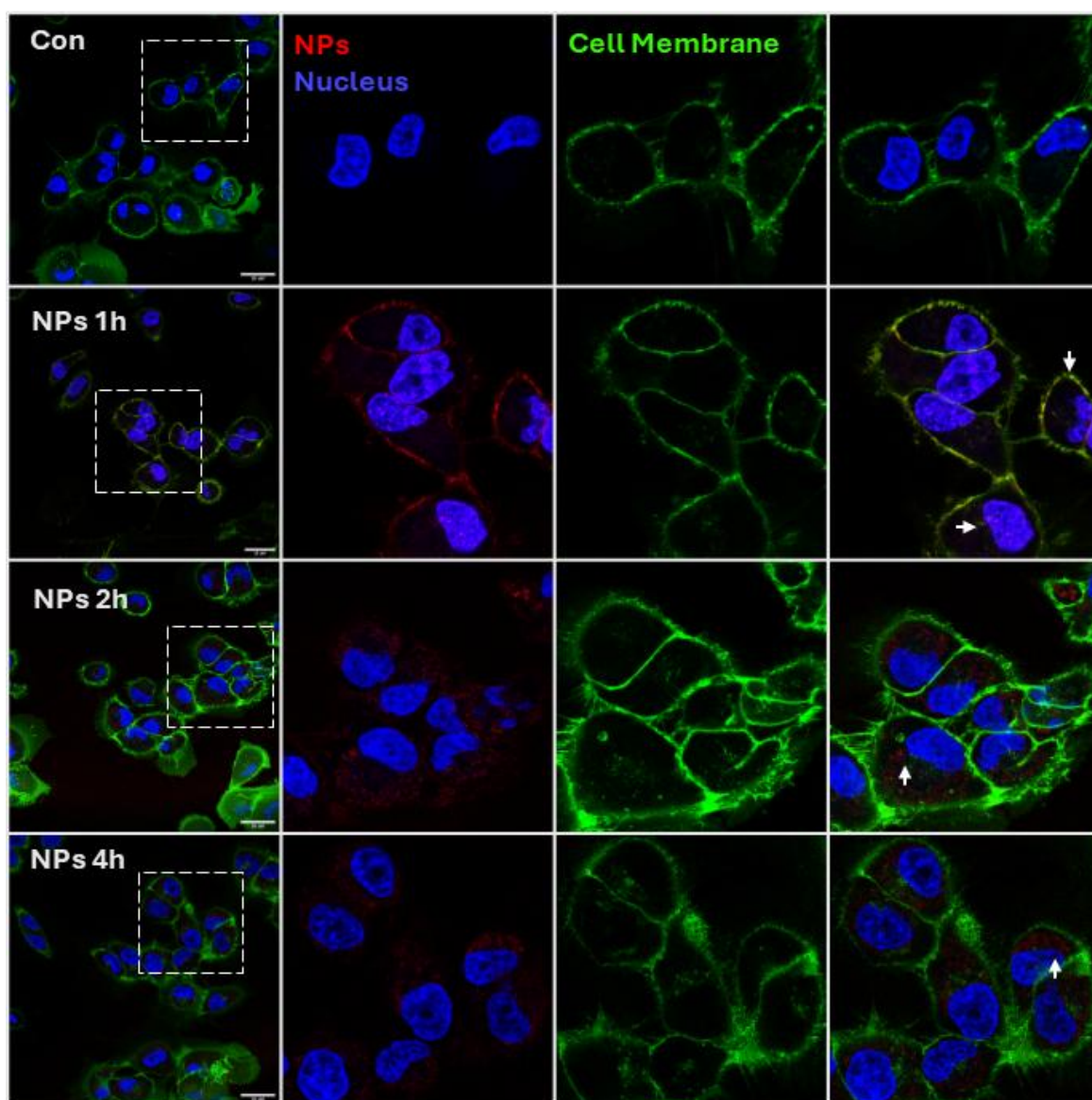


Figure 48. Internalisation of Domperidone-loaded PLGA-PEG nanoparticles (NPs) in 2D OVCAR8 cancer cells after 1h, 2h, or 4h incubation. Confocal microscopy images show cell membrane (green; CellMask), nanos (red; rhodamine), and nucleus (blue; Hoechst). Scale = 20 μm , panels 2-4 show zoomed segments (white box) with arrows (white) indicating various regions containing NPs.

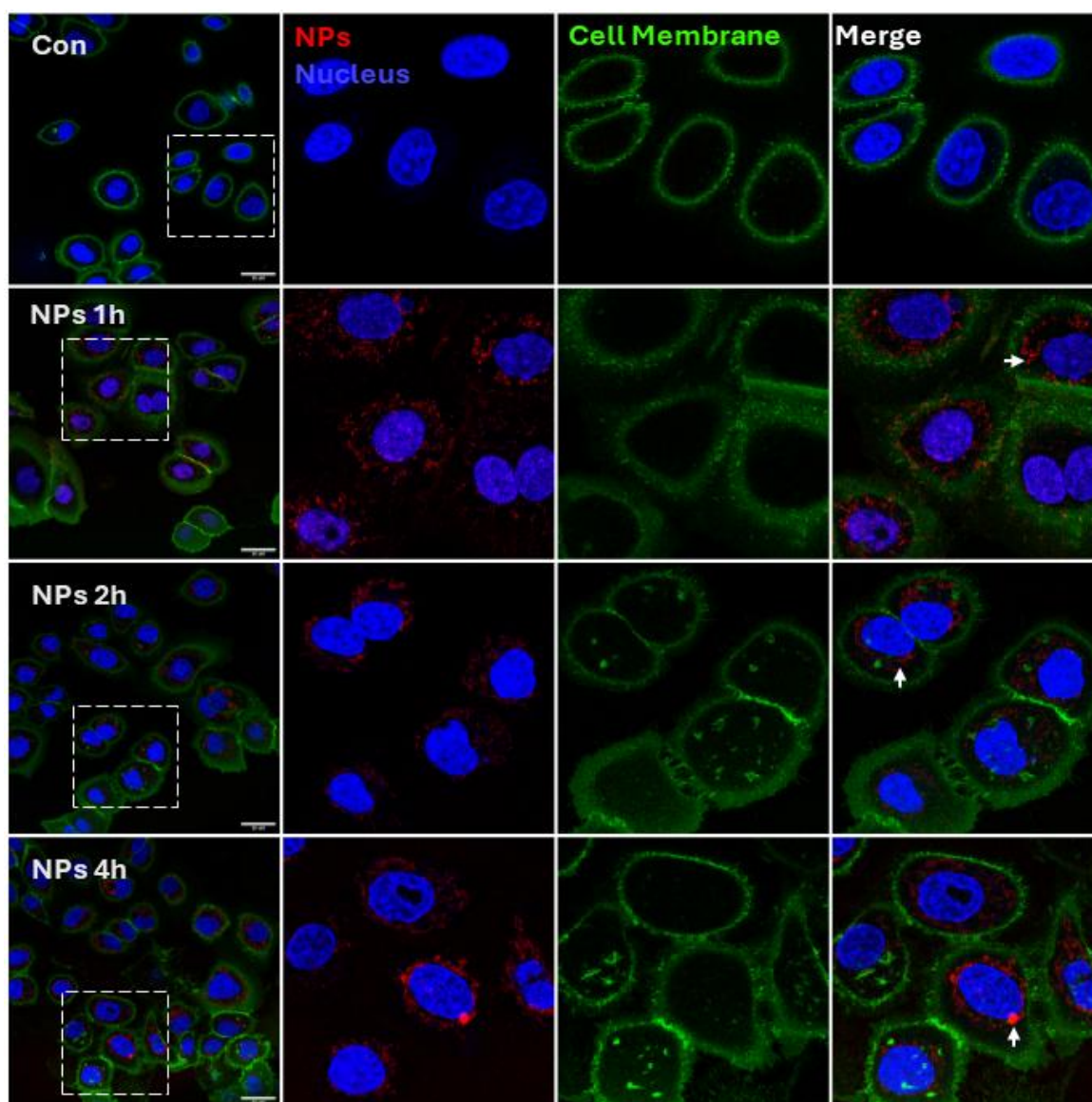


Figure 49. Internalisation of Domperidone-loaded PLGA-PEG nanoparticles (NPs) in 2D ISHIKAWA cancer cells after 1h, 2h and 4h incubation. Confocal microscopy images show cell membrane (green; CellMask), nanos (red; rhodamine), and nucleus (blue; Hoechst). Scale = 20 μm , panels 2-4 show zoomed segments (white box) with arrows (white) indicating various regions containing NPs

In contrast, 2D ISHIKAWA monolayers treated with NPs demonstrated even quicker internalisation rates. Unlike OVCAR8 cells, at 1-hour incubation, NPs were already primarily localised intracellularly in the cytoplasm and distributed around the nuclei, rather than at the cell membrane (Figure 49). Internalisation persisted after 2h, as demonstrated by small red intracellular puncta enclosed by a green-stained cell membrane in confocal microscopy images (Figure 49). Moreover, unlike OVCAR8 cells, at 4h-incubation enhanced intracellular accumulation was observed, with fluorescence appearing slightly augmented in both intensity and pixel size, which could indicate enhanced NP endocytosis and accumulation in intracellular vesicles. To confirm specificity of the red fluorescence, media-only controls stained with CellMask™, showed a total absence of red fluorescence.

5.2.7 Confocal Tracking Confirms Multicellular Uptake of NP in 3D Slice Model

To explore spatial distribution of rhodamine and Domperidone-loaded NPs, OVCAR8 OTSC slices were treated with for 2h and analysed via confocal microscopy. Initially, confocal images were taken with only a nuclear stain to ascertain whether NPs were visible within our 3D system. As seen in Figure 50, confocal images showed clear red puncta within the 3D system, indicating that our NPs could be successfully visualised. Notably, some red fluorescence could be observed near the nuclei, indicating colocalisation and possibly internalisation with cells. However, a large portion of red fluorescence was in regions of limited nuclei, indicating they were either at the cell surface or within the extracellular matrix of the TME (Figure 50).

To further explore whether the NPs colocalise with either cancer cells or immune cells, OVCAR8 slice sections were stained and imaged with the pan-epithelial marker, cytokeratin-1 (CK-1; green), or the pan-leukocyte marker CD45 (green). Confocal images demonstrate that the NPs colocalise with both cancer and immune cell within the slice. In cells stained with the pan-epithelial marker, the red NPs can be seen overlapping with the green CK-1 marker as denoted by a yellow fluorescence (white arrow; Figure 50B). As cytokeratin is a cytoplasmic marker of epithelial cells, this overlap could indicate internalisation and cytoplasmic association. However, they are also observed outside of nuclear- or cytokeratin-marked areas (red arrow; Figure 50B), which could indicate association with the extracellular matrix or cell membrane. In the slices stained with the

pan-leukocyte marker (green), the majority of the red NPs can be seen overlapping with blue-stained nuclei (white arrow; Figure 50C), with the red puncta appearing more dispersed and with smaller pixel sizes compared to the NPs that colocalise with the epithelial cell marker. As CD45 is considered a membrane-bound extracellular marker, the absence of an overlapping yellow fluorescence could indicate the NPs are not membrane-bound or within the extracellular space, but have been internalised.

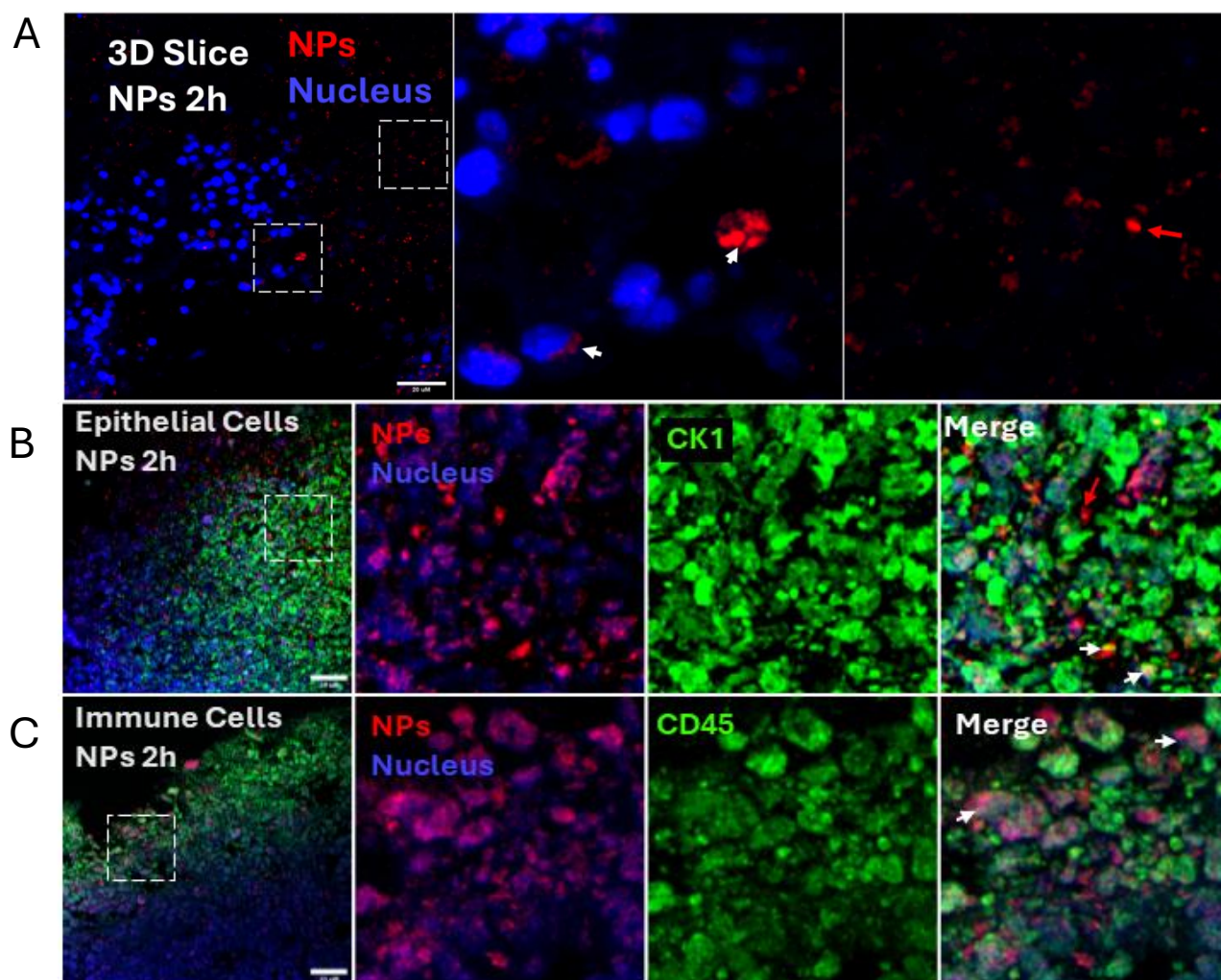


Figure 50. Spatial distribution of Domperidone-loaded PLGA-PEG nanoparticles (NPs) in 3D OVCAR8 OTSC slices after 2h incubation. **(A)** Confocal microscopy images with nanos (red; rhodamine), and nucleus (blue; Hoechst). **(B)** OVCAR8 slices counterstained with the cytoplasmic pan-epithelial marker cytokeratin-1 (CK-1; green) or **(C)** counterstained with the pan-leukocyte marker CD45 (green). Scale = 20 μ m, panels 2-4 show zoomed segments (white box) with white arrows indicating various cellular regions containing NPs, and red arrows indicating NPs in extracellular regions.

Chapter 6

Discussion

The main aim of this thesis was to develop high-fidelity *ex vivo* models of gynaecological cancers, characterise their interactomes, and validate the therapeutic potential of dopaminergic modulation. This research successfully developed slice models of HGSOC and EC and defined their tumour microenvironment and dopaminergic landscapes. Importantly, we identified the DRD2 blocker Domperidone as a promising candidate and validated its therapeutic potential in gynaecological cancer OTSC models. To address the inherent pharmacological profile of our lead candidate (Domperidone) for oncologic drug repurposing, this thesis also successfully engineered a novel PLGA-PEG nanoparticle delivery system for Domperidone.

6.1 Development of the OTSC Model: Viability

Generating reproducible OTSC models from HGSOC and EC xenografts required extensive optimisation of mechanical parameters. In agreement with others, we found that tumour consistency dictates specific blade speeds, amplitudes and frequencies (Parker et al. 2017; Weitz et al. 2022). Parker *et al.* (2017) described a similar issue that we faced, in which tumour material resided below the slicing blade cut off. Their solution was to hold the pieces vertically with forceps while they set. However, we found this proved difficult when embedding multiple tumour cores simultaneously and sometimes compromised the setting of the agarose (Parker et al. 2017). A major technical contribution of this work was the development of a dual-percentage agarose embedding technique. By leveraging a dual density block, we created an agarose support with a greater mechanical resistance to oppose the pressure of the blade, and consequently, improved slicing of softer tumours. To our knowledge, no other publications report this technique.

Regarding longevity of the slices, while we hypothesised this model would maintain viability up to 7 days, we found that slice viability declined at around Day 4 of culture. Moreover, slices sometimes exhibited metabolic fluctuations between Day 0 and Day 1, suggesting that they were undergoing a recovery phase in the first 24h post-slicing. Therefore, we concluded that drug testing should be conducted during the slice's stable phase between Day 1 and 3. This offered a 48h window, suitable for short-term *ex vivo*

drug evaluations. This aligns with Bruckner et al. (2019), who noted that while OTSC morphology can be maintained longer (up to 14 days), proliferation rates drop significantly after the first few days (Brückner et al. 2021). While OTSC offers utility for short term drug efficacy studies, it does not provide a platform to understand long-term efficacy or evolution of drug resistance mechanisms. Therefore, it is essential to extend longevity of OTSC in culture.

The lack of a functional vasculature remains a limitation of OTSC. Our study alongside others found that while plate shakers improve oxygen diffusion, the absence of active perfusion leads to a necrotic core over time (Naipal et al. 2016). One solution could be to utilise microfluidic systems termed tumour-on-chip. This system is formed of polydimethylsiloxane (PDMS) vessel-like channels that allow for constant media exchange to mimic the native functional vasculature (Lim et al. 2025). Dorigiv et al. (2021) found both the use of microfluidic platforms and micro-dissected explants (<500 µm diameter) could extend viability to 15 days (Dorigiv et al. 2021). However, with micro-dissected explants, the trade-off is a loss of spatial heterogeneity; smaller fragments may not capture the full complexity of the TME and require a higher number of replicates to achieve accurate drug response data compared to OTSC slices with a 6 mm diameter. Nevertheless, future work should investigate the incorporation of smaller fragments in a perfusable system to improve *ex vivo* slice longevity.

6.2 Development of the OTSC Model: TME Heterogeneity

The components of the TME, including immunosuppressive leukocytes and angiogenic vasculature, are critical drivers of pro-tumour biology and therapeutic resistance (Garlisi et al. 2024; Wang et al. 2025b). A primary objective of this research was to ensure the inclusion of these TME components within our *ex vivo* slice models. Our findings support this aim, demonstrating that the slices successfully retained immune and endothelial fractions at abundances comparable to the original tumour bulks. Specifically, the slice models contained a diverse repertoire of innate immune cells, including macrophages, neutrophils, and NK cells. This is in accordance with Sivakumar et al (2019) who found that their CDX OTSC slices also retained these lymphocyte and myeloid populations at

similar abundances to the original tumour bulk of breast, melanoma, and colon cancers (Sivakumar et al. 2019). However, T and B lymphocyte populations remained notably sparse in our models. This deficiency is likely attributable to the use of xenografted tumours derived from immunocompromised mice, which inherently lack mature lymphocyte populations (De Haven Brandon et al. 2020). While the absence of activated lymphocytes is a common feature in immune-cold HGSOE tumours, their total absence in these models represents a limitation in experimental fidelity (Degos et al. 2019; Kasikova et al. 2024). Moreover, while the EpCAM⁺ fraction remained stable across the endometrial slice model and original tumour bulk, we noted a decline in this population in the ovarian OTSC model. This could be due to an increase in other stromal populations, such as adipocytes or fibroblasts or downregulation of the receptor during culture and should be further investigated using a wider expansion of TME-specific biomarkers to confirm cell populations.

The cellular composition of immunocompromised xenograft tumours is heavily biased toward epithelial cancer cells, with studies showing epithelial cancer cells forming 20–60% of the entire population, whereas CD45⁺ cells contribute an average of 17–32.7% in xenografted ovarian cancer tumours (Kang et al. 2023; Steinkamp et al. 2023). Moreover, xenografted tumours primarily recruit innate host cells from the mouse. In a study of 10 ovarian patient-derived xenograft (PDX) models, macrophages were the dominant immune population in 70% of cases, while neutrophils predominated in 20% of samples (Bougherara et al. 2017). Conversely, NK cells were remarkably scarce, frequently representing less than 1% of the CD45⁺ population (Bougherara et al. 2017). These observations align with our findings, where the EpCAM⁺ population constituted 35–81% and the CD45⁺ fraction ~15%. Moreover, in accordance with the literature, the CD45⁺ fraction consisted predominantly of neutrophils (55–75%) and macrophages (30–50%) in the ovarian OTSC model, and a macrophage-dominant profile (>90%) in the endometrial OTSC model.

However, xenografted OTSC slices may be limited in representing the true immune landscape. In primary human ovarian tumours, CD45⁺ immune cells are highly variable but often constitute a dominant portion of the tumour mass, with a median frequency of 69.1% (ranging from 5% to 98%). According to Bougherara et al. (2017), tumour-infiltrating

lymphocytes represent approximately 30.5% of the immune fraction in fresh ovarian biopsies, while macrophages account for 16.2%, and neutrophils and B cells generally constitute less than 10% of the solid tumour mass (Bougherara et al. 2017).

One counteractive measure to this limitation in our study was the utilisation of patient-derived biopsy slices; these slices give an accurate snapshot of the original tumour composition of patients. While patient-derived biopsy slices offer a more immunologically representative alternative to CDX models, they introduce significant practical constraints. Tissue availability and quality were highly variable; some samples were of insufficient quantity or consistency for reliable slicing, occasionally yielding as few as four slices — sufficient for only a single metabolic assay at one drug concentration in duplicate. Furthermore, some patient slices exhibited rapid viability loss within 24–48 hours of culture, substantially limiting downstream analysis. These logistical challenges are not unique to this study and reflect a broader obstacle in the field of *ex vivo* oncology (Misra et al. 2019). Future work should prioritise establishing minimum tissue volume thresholds in surgical collection protocols and explore media optimisation or microfluidic perfusion strategies to extend patient slice longevity.

6.3 Efficacy of DRD2 Blocking (Domperidone) in Gynaecological Cancers

In this study, Domperidone treatment was efficacious in gynaecological cancer monolayers and the 3D ovarian cancer OTSC model, but not in the 3D endometrial OTSC model. This pattern mirrored findings from chemotherapy treatment, in which endometrial slices also failed to demonstrate significant cytotoxicity, suggesting that the endometrial model possesses intrinsic or microenvironment-mediated resistance mechanisms. In the ovarian OTSC model, multiple lines of evidence support a genuine cytotoxic effect. Domperidone treatment produced significant reductions in metabolic activity and proliferation, increased apoptotic staining, and confocal imaging confirmed a substantial reduction in viable cell populations. While lactate dehydrogenase release showed an increasing trend, it did not reach statistical significance, likely reflecting limitations in assay sensitivity and high biological variation rather than a true absence of cell damage. In contrast, the endometrial OTSC model exhibited minimal cytotoxic

response across metabolic, IHC, and confocal assessments despite demonstrating 2D efficacy, with proliferation significantly increasing and apoptotic staining showing a non-significant reduction following Domperidone treatment.

The divergence between 2D and 3D results highlights the importance of tissue context in modulating drug sensitivity, as has been widely reported in organoid-based systems. When ovarian cancer cell lines were cultured in 2D monolayers, they were up to 280-fold more sensitive to chemotherapy than the same lines grown in a 3D organoid format (Brodeur et al. 2021). Similarly, HGSOC organoids containing only cancer cells were more sensitive to chemotherapy than tri-component organoids grown with CAFs and macrophages, underscoring the role of TME elements in drug resistance (Burkhard et al. 2025). The reduced sensitivity observed in 3D models likely reflects a combination of factors, including limited drug penetration and enhanced cell–cell and cell–matrix (Santo et al. 2017). Notably, the greater cytotoxicity observed in 2D OVCAR8 cells compared to the ovarian OTSC model may similarly reflect the presence of immune and endothelial cells in the 3D model that remain metabolically active and proliferating, thereby diluting the apparent drug response.

In fresh human ovarian cancer patient samples, response to DRD2 blockade was heterogeneous. Three patients (two HGSOC and one mucinous) responded extremely strongly, and one HGSOC patient responded partially to Domperidone, while borderline and endometrioid ovarian cancer patients showed limited to no response. Although the sample size was small and overall efficacy demonstrated a trend rather than a statistically significant change, these findings suggest that treatment response may be subtype-dependent. This is consistent with other groups using gynaecological OTSC slices from patients who report patient specific drug responses (Estes et al. 2007; Frederick et al. 2009; Lheureux et al. 2015; Brückner et al. 2021).

Interestingly, analysis of available datasets revealed that DRD2 expression was associated with improved prognosis in ovarian cancer, whereas previously we found DRD2 expression was associated with worse prognosis in endometrial cancer (Español et al. 2023); this pattern that may relate to our experimental findings, in which the endometrial model exhibited high DRD2 expression yet resistance to DRD2 antagonism, while the ovarian OTSC model showed both high DRD2 expression and strong efficacy to

Domperidone. Further patient samples will need to be tested alongside quantification of DRD2 expression to fully ascertain which subtype-specific ovarian cancer populations would likely benefit from DRD2 blockade, and to evaluate the utility of DRD2 as a prognostic marker.

6.4 Signalling Modulation by DRD2 Blocking (Domperidone)

The efficacy of Domperidone is likely due to dopaminergic-mediated alterations in downstream signalling cascades. Signalling of DRD2 through the β -arrestin-2 dependent pathway is associated with increased Raf/MEK/ERK signalling (Urs et al. 2016; Han et al. 2019). Blocking of this receptor is known to cause the downregulation of DRD2/ β -arrestin-2 complex through receptor endocytosis and endosomal trafficking, perturbing the phosphorylation of the downstream effectors p-MEK and p-ERK (Tung et al. 2022). Multiple studies report both p-MEK and p-ERK are downregulated following Domperidone treatment in oesophageal squamous cell carcinoma and colorectal cancer cell lines (Sim et al. 2024; Yuan et al. 2024). Sim et al. (2024) observed a reduction in DRD2 expression, proposing this event as the mechanism for p-ERK downregulation and Domperidone-induced cytotoxicity. However, Yuan *et al.*, (2024) hypothesised a direct intracellular inhibitory effect of Domperidone. Their *in-silico* docking analyses predicted that Domperidone could directly bind to three kinases (MEK1, MEK2 and CDK4). The authors also confirmed MEK-Domperidone binding through pulldown assays, which could be reversed through mutating the kinases. Thus, proposing Domperidone disrupts MEK/ERK and CDK4/SMAD3 signalling pathways due to direct inhibition (Yuan et al. 2024).

In line with these results, we too found DRD2 downregulation at the protein level in both OVCAR8 2D cells and 3D OTSC models. Moreover, β -arrestin-2 was also downregulated alongside decreased levels of MEK, p-MEK and ERK. However, paradoxically, phosphorylated ERK levels increased. Therefore, it is likely an alternative pathway is being activated to induce ERK activation. For instance, through PI3K/AKT mediated MEK-independent pathways (Aksamitiene et al. 2010). This should be confirmed with mRNA quantification or complementary protein-based assays. Interestingly, another DRD2

antagonist, ONC201, triggers p53-independent apoptosis by suppressing MCL-1 and increasing death receptor 5 (DR5) expression. Moreover, its dual-dephosphorylation of AKT and ERK pathways were shown to increase the expression of apoptotic effector bim and the transcription of the DR5 ligand and TRAIL, consequently triggering apoptosis (Ishizawa et al. 2015; Tu et al. 2017; Fang et al. 2018). This could be a possibility in our models; both OVCAR8 and ISHIKAWA cells contain mutant p53 and showed significant cytotoxicity when treated with our DRD2 blockade. Therefore, future experiments should investigate Domperidone's effects on apoptotic effectors (MCL-1, bim, bad) and TRAIL components to fully ascertain the anti-cancer mechanism.

Like Yuan et al. (2024), we also investigated whether the mechanism of Domperidone was extracellular or intracellular through co-administration of Domperidone with endocytosis inhibitors. We found that inhibitors specific for macropinocytosis, caveolae-mediated, and clathrin-mediated endocytosis did not significantly reduce or prevent Domperidone-mediated cytotoxicity. While Colchicine, a pinocytosis and phagocytosis inhibitor, did exhibit a significant increase in viability in Domperidone-treated cells, the inhibitor alone was also significantly cytotoxic; this could explain the result, in which two cytotoxic drugs can have antagonistic interactions (Chen et al. 2025). However, we cannot completely rule out pinocytosis of Domperidone. Therefore, future experiments should investigate Domperidone efficacy with another pinocytosis inhibitor which does not cause significant cytotoxicity.

6.5 Compensatory Mechanisms

(a) OVCAR8 OTSC Model

While efficacious, the pharmacological potency of Domperidone was reduced in 3D ovarian OTSC model compared to the 2D monolayer, potentially indicating an underlying compensatory mechanism. This is likely centred around DRD1 upregulation and persistent p-ERK, despite downregulation upstream effectors, and the AKT pathway. For example, transcriptomic and proteomic quantification revealed downregulation of DRD2 with a paradoxical increase in DRD1 and DRD5. Moreover, we found that p-AKT levels increased and p-GSK3 remained stable, and p-MEK levels decreased while p-ERK levels

rose. Together, these findings suggest that in the OVCAR8 OTSC model, the upregulation of DR1-like pathways likely triggers the canonical $G_{\alpha s}$ /cAMP/PKA or PI3K/AKT axis leading to the sustained phosphorylation of ERK and AKT/GSK3 β .

Phosphorylation of GSK3 β leads to stabilised β -catenin – a fundamental player in the Wnt signalling pathway and associated with the upregulation of stem-like genes, such as CD24, CD44, PROM1, EPCAM, CXCR4 and CD133 (Kim and Kahn 2014; Han et al. 2019). We saw a concentration-dependent upregulation of all these genes in Domperidone-treated OVCAR8 slices. Therefore, it is likely that the wnt/ β -catenin pathway is being upregulated as a compensatory mechanism. Critically, the efficacy of this signalling compensation is likely amplified by the specific genetic background of the OVCAR8 line. The presence of CTNNB1 (β -catenin) missense mutations within the NH₂-terminal regulatory domain renders the protein resistant to the GSK3 β /Axin/APC destruction complex (Rask et al. 2003; Krishna et al. 2026). This compensatory mechanism could be preserved or potentially enhanced by the 3D microenvironment, creating a Wnt pathway-bypass that allows the tumour to maintain cancer stem cell gene expression and some level of proliferation, despite clearly demonstrating DRD2 blockade-induced apoptosis. This was a similar finding to Yamamoto *et al.* (2019) who found Wnt signalling was a bypass route to resist olaparib therapy in *ex vivo* ovarian cancer slices. Subsequently, they found an increased efficacy of PARPi therapy with co-administration of the FDA-approved Wnt signalling inhibitor, pyrvinium pamoate (Yamamoto et al. 2019). This poses the question whether DRD2 antagonism would be synergistically potent when administered alongside a β -catenin-targeted agent.

Interestingly, the GPCR ADRA1A was also upregulated in ovarian OTSC after Domperidone treatment, and high expression of this gene has been linked to increased adipocyte thermogenesis, metabolism, and worse survival in cancer (Wang et al. 2019; Rahbani et al. 2022). Thus, ADRA1A could also be a pro-tumour compensation.

(b) ISHIKAWA OTSC Model

The strong pharmacological resistance to Domperidone observed in the 3D endometrial models in contrast to the sensitivity of 2D monolayers highlights a potent molecular bypass mechanism. In endometrial cell lines, a downregulation of β -Arrestin, AKT, GSK-

3 β , MEK, p-MEK, and p-ERK were observed. Interestingly, GSK3 β has been shown to regulate actin cytoskeleton dynamics and microtubule formation to drive cell migration (Sun et al. 2009; Guo et al. 2019). GSK3 β was downregulated in 2D ISHIKAWA cells but not in 2D OVCAR8 cells after Domperidone treatment, moreover more EMT-associated genes were upregulated in the OVCAR8 model than the endometrial model; these genes (e.g., CXCR4, CD133, CD44) are associated with migratory stem cell-like behaviours (Cioffi et al. 2015; Zhao et al. 2020). This may explain the differences in migratory behaviour during the wound repair assay, in which ISHIKAWA cells treated with Domperidone had significantly less wound closure than the control, whereas OVCAR8 cells remained unchanged.

Differences between the two cell lines also appeared in signalling mechanisms; while p-ERK was slightly downregulated and p-AKT slightly upregulated in the ISHIKAWA cells treated with Domperidone, the exact opposite occurred in the OVCAR8 cell line model, indicating that our endometrial cell line has an intrinsic reliance on the AKT pathway. Interestingly, ISHIKAWA has PI3KA gain-of-function and PTEN loss-of-function mutations which are key players in the regulation of the PI3KA/AKT pathway, leading to phosphorylation of AKT (Vidal-Sabanés et al. 2025). It could be that this mutated overactivated pathway is utilised in the 3D endometrial model to resist therapy. However, we did not explore protein alterations in the 3D OTSC system, so we cannot further speculate on the specific compensatory mechanism. In transcriptomic analysis, we also observed an increase in the receptor tyrosine kinase Kit, alongside upregulation of the GCPR ADRA1B, known to be upregulated by NE expression causing increased cell growth and proliferation through PI3K/AKT pathways (Vandenput et al. 2011). Therefore, it is possible that the ISHIKAWA 3D slices are utilising other receptor pathways to resist Domperidone, but this would need further exploration.

6.6 Domperidone and TME Effects

Interestingly, distinct cellular and molecular changes in the TME occurred between the two cancers following Domperidone treatment that could give us an insight into drug resistance mechanisms. Firstly, Domperidone treatment induced distinct immune responses across tumour types; in ovarian slices, a significant trend of increased macrophage markers and decreased neutrophil markers was observed, whereas

endometrial slices exhibited increases across multiple immune populations, including macrophages, neutrophils, and natural killer cells. Despite these changes, overall CD45-positive cell levels remained relatively stable, suggesting that Domperidone primarily modulates immune cell phenotype rather than overall abundance.

(a) Macrophages

Domperidone induced an increase in F4/80+ cells (macrophages), however the macrophage-specific Ly6C marker was downregulated at the mRNA level in the 3D ovarian cancer model. This could be indicative of a shift in macrophage polarisation, potentially towards a more differentiated or repair-associated phenotype. This may be linked to compensatory activation of DRD1 signalling, which has been associated with anti-inflammatory M2 macrophage polarisation via PKA/CREB pathway (Liu et al. 2021a). One study found that DA-DRD2 signalling induces M2-to-M1 polarisation that could be blocked by DRD2 antagonism (Qin et al. 2015). Conversely, others show the opposite, with DRD2 expression downregulated in M1 macrophages, DRD2 agonistic signalling driving pro-tumour M2 phenotypes, and DRD2 antagonism selectively blocking YAP-dependent fibrogenesis (characteristic of M2 macrophages) (Qing et al. 2022; Fan et al. 2023). Therefore, DRD2 antagonism and receptor downregulation could play a role in anti-tumour M1 polarisation. While observable DRD1 upregulation and DRD2 downregulation occurred, it is unclear of the specific dopaminergic modulation and polarisation that is occurring in macrophages within our 3D OTSC system. Therefore, it would be interesting to conduct *in vitro* experiments studying macrophage viability and polarisation following Domperidone treatment. We could then extend this analysis to the 3D OTSC system to explore the impact of the TME on this process.

(b) Neutrophils

Interestingly, neutrophil responses were the opposite in the two gynaecological OTSC slice models; while ovarian cancer slices demonstrated signs of neutrophil downregulation, endometrial cancer slices exhibited increased neutrophil abundance. DRD2 antagonism has been associated with increased neutrophil inflammation, whereas DRD1 agonism induces neutrophil apoptosis (Sookhai et al. 1999). We know

that DRD1 protein and mRNA is increased in the ovarian model but not in endometrial cell line and 3D model mRNA. Therefore, one explanation could be neutrophil stimulation is being driven by initial DRD2-blocking in endometrial models but downregulated in ovarian cancer models by a DRD1 compensation. Interestingly, we also found that in the ovarian OTSC model, the DRD2+ cells were mostly immune cells, whereas in endometrial OTSC they were mostly epithelial cells. This discrepancy could suggest that the immune cells of the ovarian OTSC platform are more responsive to DRD2 antagonism, than the endometrial OTSC interactome.

(c) NK cells

DRD1 agonists increase NK cell-mediated cytotoxicity of lymphoma cells via the cAMP/PKA/CREB pathway, whereas DRD2 stimulation is a potent immunosuppressor of NK cells (Zhao *et al.*, 2013). In our study we found that OVCAR8 3D OTSC slices exhibited a non-significant upward trend of NK1.1+ cells after 48h with increasing Domperidone concentrations. Moreover, endometrial OTSC slices also demonstrated a significant dose-dependent increase in NK cells after 24h which stabilised at 48h. Therefore, it is possible that Domperidone is driving NK cell activation, but this requires further investigation. Moreover, the dissipation of NK cell stimulation at 48h post-therapy and lack of cytotoxicity in the endometrial cancer could be suggesting that there is an underlying TME-mediated compensatory mechanism that is driving immunosuppression and drug resistance.

(d) Endothelial Cells

DRD2 blocking may also be influencing vascular-associated processes in the ovarian cancer OTSC model. It has been reported that DRD2 expression is upregulated in tumour associated endothelial cells compared to the regular endothelium as a response to increased VEGF-A mediated signalling. However, an increase in DRD2 receptor expression and agonistic signalling is associated with decreased VEGFR expression and reduced endothelial cell proliferation and migration (Basu *et al.* 2001; Sarkar *et al.* 2022). Conversely, DRD2 antagonists have been implicated in VEGF and VEGFR2 downregulation in HGSOE and EC, inhibiting cancer adhesion, invasion, and growth (Fang *et al.* 2018; Tucker *et al.* 2022).

Our study found that both gynaecological 3D models contained DRD2+ endothelial cells, and that DRD2 blocking by Domperidone significantly increased endothelial cell abundance in the ovarian OTSC model. Conversely, the ovarian OTSC model also had significant dose-dependent decreases of CD31 mRNA expression. It is possible that DRD2 antagonism is causing a downregulation of CD31 gene transcription due to a recent upregulation of CD31 translation and protein trafficking at the endothelial cell surface. As CD31 is a tight junction protein, protein increases could be related to the normalisation of leaky tumour vasculature. However, it could also be a sign of endothelial cell proliferation and angiogenic signalling (Tang et al. 2019; Sun et al. 2023). Therefore, further investigations are required to study the effect of Domperidone on endothelial cells through the incorporation of pericyte markers, such as α -SMA or NG2, which would typically increase during vasculature normalisation (Moreno-Smith et al. 2013).

6.7 Domperidone Loaded PLGA-PEG Nanoparticles

To address the pharmacological challenges of repurposing Domperidone for oncological use, specifically its poor solubility, opsonisation and potential off-target effects, this study evaluated a novel PLGA-PEG nanoparticle delivery system. While Domperidone-loaded nanocarriers demonstrated some cytotoxicity in the 2D endometrial cancer model, overall, the NPs exhibited lower therapeutic efficacy than the free drug in both 2D and 3D models.

Several factors likely contribute to this reduced potency. Firstly, the effective concentration of Domperidone loaded in PLGA-PEG carrier may have been sub-therapeutic. It has been reported that single material polymers have limited loading capacity (Halder et al. 2025). Our filtered NPs achieved an average of 34.2 $\mu\text{g/mL}$ (80 μM), however the NPs were used at a 1:10 dilution in media and therefore, the effective concentration was $\sim 8 \mu\text{M}$ which is 5 to 9-fold lower than our *ex vivo* efficacy threshold (35-70 μM). Therefore, loading more into the nanoparticles is essential. Alternative formulations in the literature, such as mixed polymeric micelles or lipid nano-formulations, have achieved significantly higher Domperidone loading (300-1000 $\mu\text{g/mL}$), with encapsulation efficiencies of 87.8-90.5% (Thatipamula et al. 2011; Halder et al.

2025). Beyond materials, greater encapsulation efficiencies could also be achieved by using a greater mass of nanomaterial, employing a ‘hot’ oil-in-water nanoprecipitation method, adding surfactant (2% Tween), or using organic solvents with higher drug-solubility profiles, such as chloroform (Thatipamula et al. 2011; Halder et al. 2025). Furthermore, the absence of characterised release kinetics leaves it unclear whether the reduced efficacy was also a result of drug retention within the polymer matrix. Future iterations of this protocol should focus on optimising the drug-loading efficiency and characterise the release kinetics.

Importantly, our mechanistic findings suggest that Domperidone-mediated cytotoxicity and DRD2 downregulation primarily stems from extracellular receptor binding. If the nanoparticle formulation exhibits rapid intracellular internalisation prior to drug release, it effectively bypasses the necessary membrane-interaction phase. Our 3D OTSC tracking showed that while some NPs remained in the interstitial spaces of the stroma, the majority were internalised by cancer and immune cells, likely preventing the downregulation of the receptor and canonical DRD2-signalling pathway.

This result necessitates a shift in nanoparticle design towards environmentally-sensitive, extracellular release strategies. One such approach is the development of a multifunctional pH-responsive delivery system. For instance, Wang et al. (2021) demonstrated that by utilising pH-labile covalent linkers, a vascular-targeting prodrug (Combretastatin A4) could be selectively released into the acidic tumour microenvironment at pH ~6.7 before the remaining carrier was internalised via folate-mediated targeting (Wang et al. 2021). Implementing a similar spatiotemporal controlled release for Domperidone in the TME while the particle is still extracellular could restore its therapeutic potency by ensuring maximum interaction with the membrane-bound DRD2 receptors. Alternatively, DRD2 could be used as a target using surface-conjugated peptides or affibodies. Conjugating a DRD2-targeting peptide to the outside of a nanocarrier could encourage DRD2 internalisation and endocytic trafficking of both receptor and the nanocarrier. This nanocarrier could therefore encapsulate a synergistic therapeutic, for example an antagonist of the Wnt or AKT pathways which we hypothesised as the potential bypass mechanisms during DRD2 blockades.

Lastly, while efficacy was limited in our models, the 3D OTSC platform proved to be a highly valuable tool for assessing the biodistribution and toxicology of nano-formulations in a complex tissue architecture. We found that the NPs appeared to demonstrate multicellular uptake within the 3D tumour slice, observing internalisation by both malignant epithelial cells and infiltrating immune cells. While confocal microscopy provided clear evidence of association, precisely defining and quantifying the NP uptake remains a challenge. Previous studies, such as Bartucci et al. (2020), utilised tissue disaggregation and flow cytometry in *ex vivo* liver slices to characterise population-specific uptake and quantify uptake percentages; however, such methods sacrifice the critical spatiotemporal context of nanoparticle penetration (Bartucci et al. 2020). Nevertheless, integrating flow cytometry with our established confocal analysis could offer a powerful, complementary approach for future studies. To our knowledge, this is the first study to characterise nanoparticle tracking in *ex vivo* gynaecological cancer slices, and could provide a useful model for drug penetration, biodistribution and cytotoxicity studies.

6.8 Limitations and Future Directions

Through the discussion we have mentioned various limitations and future directions we would like to implement, including improving OTSC viability through advanced microfluidic systems or smaller tissue explants, characterising the TME further with additional biomarkers specific for fibroblasts, M1/M2 macrophages, cytotoxic NK/T cells, and pericytes, and utilising additional assays and markers of signalling proteins in the PI3K/AKT, MAPK and TRAIL/apoptosis pathways to fully understand the effects of DRD2 blocking on downstream processes. To refine our nanodelivery strategy, future studies could explore the use of pH-sensitive linkers or surface-conjugated peptides specific for DRD2 to encourage extracellular DRD2 blocking. Furthermore, given the emergence of Wnt-pathway and AKT-mediated resistance identified in our OTSC models, these nanoparticles could serve as a versatile multi-targeting platform. Encapsulating a synergistic secondary agent, such as a Wnt inhibitor or PI3K/AKT antagonist, alongside a DRD2-targeting shell could potentially overcome the compensatory mechanisms identified in this thesis.

Chapter 7

Conclusions

Conclusions

- 1. Successful Development of a Novel *Ex vivo* Tumour Slicing and Culture Model of Gynaecological Cancers.** We established a reproducible protocol for preparing Organotypic Tumour Slice Cultures (OTSC) that preserved the native tumour microenvironment (TME), including crucial immune cell populations such as macrophages, neutrophils, and NK cells. We introduced methodical innovations by embedding samples in varying densities of agarose. This advancement enabled high-quality slicing and maintained tissue integrity.
- 2. Establishment of an Optimal 48-Hour Drug Testing Window.** We determined OTSC remained viable and metabolically stable between Day 1 and Day 3 post-slicing. This 48-hour window offers a reliable timeframe to conduct short-term, high-fidelity drug efficacy screens within an *ex vivo* environment reflective of *in vivo* human tumours.
- 3. Superior Predictive Value of 3D OTSC Over Traditional 2D Cultures.** Comparative drug response studies with traditional 2D cultures and 3D OTSC revealed that while both Domperidone and standard chemotherapeutics were broadly cytotoxic in 2D monolayer cultures, their efficacy within OTSC was highly context-dependent.
- 4. DRD2 Blocking Efficacy in HGSOC model but not EC Model.** While therapeutics were potent in high grade serous ovarian cancer, they were largely ineffective in endometrial cancer. These findings reinforce the superiority of 3D *ex vivo* models and the inclusion of the TME for accurately forecasting clinical drug resistance.
- 5. Signalling Paradoxes Under DRD2 Blockade.** Mechanistic analyses demonstrated that although Domperidone successfully inhibited the canonical DRD2/ β -arrestin-2 and MEK/p-MEK pathways, a paradoxical increase in phosphorylated ERK (p-ERK) was observed. This suggests that tumours can activate alternative compensatory survival signalling routes when challenged with DRD2 antagonists. Moreover, through endocytosis inhibition assays, we concluded that Domperidone likely acts through

extracellular binding and receptor trafficking rather than intracellular kinase inhibition.

6. Identification of Alternative Resistance Pathways and Tumour Plasticity. Ovarian tumours upregulated DRD1/5 and stem cell markers alongside CTNNB1 (β -catenin) mutations, implicating a DRD1/Wnt/ β -catenin-mediated bypass. Whereas endometrial tumours exhibited increased expression of KIT (TRK) and ADRA1B, and PTEN/PI3K mutations, indicating a distinct molecular route that sustains phosphorylated AKT activity.

7. Elucidation of Neuromodulatory Effects on the Tumour Microenvironment. Domperidone exerted diverse immune-modulating effects, stimulating NK cells and modulating macrophages across tumour types. Notably, DRD2 blocking suppressed neutrophils in HGSOc yet promoted them in EC. In ovarian models, the DRD2 receptors were primarily on immune cells, whereas in endometrial models, they were on epithelial cells, this could explain the divergent responses, reflecting distinct receptor distributions and complexity of neurotransmitter signalling in the TME.

8. Evidence for Vascular Normalisation Following DRD2 Antagonism. Analysis of vascular markers revealed that Domperidone reduced CD31 expression but increased overall endothelial cell abundance. While endothelial modulation is observed, it remains unclear whether DRD2 blockade contributes to pro- or anti-tumour angiogenic signalling.

9. Validation of OTSC as a Platform for Nanomedicine Development. Utilising engineered Domp-PLGA-PEG nanoparticles demonstrated the applicability of OTSC for nanomedicine biodistribution and efficacy studies. Although initial therapeutic performance was low, likely due to drug delivery challenges, these *ex vivo* models provide an essential bridge for translational drug development prior to *in vivo* experimentation.

References

1. Abdolahi, S., Ghazvinian, Z., Muhammadnejad, S., Saleh, M., Asadzadeh Aghdaei, H., & Baghaei, K. (2022). Patient-derived xenograft (PDX) models, applications and challenges in cancer research. *Journal of Translational Medicine* 2022 20(1), 206 <https://doi.org/10.1186/s12967-022-03405-8>
2. AbuShahin, F., Rose, P. G., & for correspondence, A. (2007). Drug Evaluation: Role of gemcitabine in the treatment of ovarian cancer. *Women's Health*, 3(3), 279–290. <https://doi.org/10.2217/17455057.3.3.279>
3. Ahmadi-Nouraldinvand, F., Bourang, S., Azizi, S., Noori, M., Noruzpour, M., & Yaghoubi, H. (2024). Preparation and characterization of multi-target nanoparticles for co-drug delivery. *Medicine in Drug Discovery*, 21, 100177. <https://doi.org/10.1016/j.medidd.2024.100177>
4. Ahmed, E. N., Cutmore, L. C., & Marshall, J. F. (2024). Syngeneic Mouse Models for Pre-Clinical Evaluation of CAR T Cells. *Cancers* 2024, Vol. 16, 16(18). <https://doi.org/10.3390/cancers16183186>
5. Åkesson, Å., Adok, C., & Dahm-Kähler, P. (2023). Recurrence and survival in endometrioid endometrial cancer - a population-based cohort study. *Gynecologic Oncology*, 168, 127–134. <https://doi.org/10.1016/j.ygyno.2022.11.012>
6. Aksamitiene, E., Kholodenko, B. N., Kolch, W., Hoek, J. B., & Kiyatkin, A. (2010). PI3K/Akt-sensitive MEK-independent compensatory circuit of ERK activation in ER-positive PI3K-mutant T47D breast cancer cells. *Cellular Signalling*, 22(9), 1369–1378. <https://doi.org/10.1016/j.cellsig.2010.05.006>
7. Al Habyan, S., Kalos, C., Szyborski, J., & McCaffrey, L. (2018). Multicellular detachment generates metastatic spheroids during intra-abdominal dissemination in epithelial ovarian cancer. *Oncogene* 2018 37:37, 37(37), 5127–5135. <https://doi.org/10.1038/s41388-018-0317-x>
8. Alam, S. K., Pandit, A., Wang, L., Mortazavi Farsani, S. S., Thiele, B. A., Manoj, P., Aubry, M. C., Verma, V., Rudin, C. M., Lo, Y. C., & Hoepfner, L. H. (2025). Dopamine D2 receptor agonists abrogate neuroendocrine tumour angiogenesis to inhibit chemotherapy-refractory small cell lung cancer progression. *Cell Death & Disease* 2025 16:1, 16(1), 370-. <https://doi.org/10.1038/s41419-025-07693-y>
9. Amin, M., Huang, W., Seynhaeve, A. L. B., & Ten Hagen, T. L. M. (2020). Hyperthermia and Temperature-Sensitive Nanomaterials for Spatiotemporal Drug Delivery to Solid Tumors. *Pharmaceutics*, 12(11), 1–23. <https://doi.org/10.3390/pharmaceutics12111007>
10. Andrade, D. A. P., Bonatelli, M., de Paula, F. E., Berardinelli, G. N., Teixeira, G. R., dos Reis, M. T., Barbin, F. F., Andrade, C. E. M. da C., Aguiar, V. P., Hermoza, A. D., Hirai, W. Y., Schmidt, R. L., Reis, R. M., & dos Reis, R. (2024). Implementation of the ProMISE classifier and validation of its prognostic impact in Brazilian endometrial carcinomas. *Frontiers in Oncology*, 14, 1503901. <https://doi.org/10.3389/fonc.2024.1503901>
11. Ariyanayagam, S., & Baksh, D. (2024). P53 Abnormal Endometrial Cancers – Analysis of PD-1 Co-Expression by CD4 helper and CD8 Cytotoxic T-Cell Subtypes in the Tumor

- Microenvironment. *American Journal of Clinical Pathology*, 162(Supplement_1), S96–S96. <https://doi.org/10.1093/ajcp/aqae129.214>
12. Arora, S., Balasubramaniam, S., Zhang, H., Berman, T., Narayan, P., Suzman, D., Bloomquist, E., Tang, S., Gong, Y., Sridhara, R., Turcu, F. R., Chatterjee, D., Saritas-Yildirim, B., Ghosh, S., Philip, R., Pathak, A., Gao, J. J., Amiri-Kordestani, L., Pazdur, R., & Beaver, J. A. (2021). FDA Approval Summary: Olaparib Monotherapy or in Combination with Bevacizumab for the Maintenance Treatment of Patients with Advanced Ovarian Cancer. *The Oncologist*, 26(1), e164–e172. <https://doi.org/10.1002/onco.13551>
 13. Atiya, H., Frisbie, L., Pressimone, C., & Coffman, L. (2020). Mesenchymal Stem Cells in the Tumor Microenvironment. *Advances in Experimental Medicine and Biology*, 1234, 31–42. https://doi.org/10.1007/978-3-030-37184-5_3
 14. Atiya, H. I., Gorecki, G., Garcia, G. L., Frisbie, L. G., Baruwat, R., & Coffman, L. (2023). Stromal-Modulated Epithelial-to-Mesenchymal Transition in Cancer Cells. *Biomolecules* 2023, Vol. 13, Page 1604, 13(11), 1604. <https://doi.org/10.3390/BIOM13111604>
 15. Attané, C., & Muller, C. (2020). Drilling for Oil: Tumor-Surrounding Adipocytes Fueling Cancer. *Trends in Cancer*, 6(7), 593–604. <https://doi.org/10.1016/j.trecan.2020.03.001>
 16. Axemaker, H., Plesselova, S., Calar, K., Jorgensen, M., Wollman, J., & de la Puente, P. (2024). Reprogramming of normal fibroblasts into ovarian cancer-associated fibroblasts via non-vesicular paracrine signaling induces an activated fibroblast phenotype. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1871(7), 119801. <https://doi.org/10.1016/j.bbamcr.2024.119801>
 17. Azevedo, R. G. M. V., Harada, G., Da Costa, S. C. S., Miranda, V. C., De Freitas, D., Filho, E. A., De Oliveira Ferreira, P. A., Gabrielli, F., Diz, M. D. P. E., & Da Cunha Colombo Bonadio, R. R. (2018). Adjuvant Carboplatin and Paclitaxel Chemotherapy Followed by Radiotherapy in High-Risk Endometrial Cancer: A Retrospective Analysis. *Journal of Global Oncology*, 4(4), 1–8. <https://doi.org/10.1200/JGO.17.00146>
 18. Baka, Z., Godier, C., Lamy, L., Mallick, A., Gribova, V., Figarol, A., Bezdetnaya, L., Chateau, A., Magne, Z., Stiefel, M., Louaguef, D., Lavalle, P., Gaffet, E., Joubert, O., & Alem, H. (2023). A Coculture Based, 3D Bioprinted Ovarian Tumor Model Combining Cancer Cells and Cancer Associated Fibroblasts. *Macromolecular Bioscience*, 23(3), 2200434. <https://doi.org/10.1002/MABI.202200434>;WGROU:STRING:PUBLICATION
 19. Balasubramaniam, S., Beaver, J. A., Horton, S., Fernandes, L. L., Tang, S., Horne, H. N., Liu, J., Liu, C., Schrieber, S. J., Yu, J., Song, P., Pierce, W., Robertson, K. J., Palmby, T. R., Chiu, H. J., Lee, E. Y., Philip, R., Schuck, R., Charlab, R., ... Pazdur, R. (2017). FDA Approval Summary: Rucaparib for the Treatment of Patients with Deleterious BRCA Mutation-Associated Advanced Ovarian Cancer. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, 23(23), 7165–7170. <https://doi.org/10.1158/1078-0432.CCR-17-1337>

20. Barnes, B. M., Nelson, L., Tighe, A., Burghel, G. J., Lin, I.-H., Desai, S., McGrail, J. C., Morgan, R. D., & Taylor, S. S. (2021). Distinct transcriptional programs stratify ovarian cancer cell lines into the five major histological subtypes. *Genome Medicine*, 13(1), 140. <https://doi.org/10.1186/s13073-021-00952-5>
21. Barr, J. L., Kruse, A., Restaino, A. C., Tulina, N., Stuckelberger, S., Vermeer, S. J., Williamson, C. S., Vermeer, D. W., Madeo, M., Stamp, J., Bell, M., Morgan, M., Yoon, J. Y., Mitchell, M. A., Budina, A., Omran, D. K., Schwartz, L. E., Drapkin, R., & Vermeer, P. D. (2021). Intra-tumoral nerve-tracing in a novel syngeneic model of high-grade serous ovarian carcinoma. *Cells*, 10(12), 3491. <https://doi.org/10.3390/CELLS10123491/S1>
22. Bartucci, R., Åberg, C., Melgert, B. N., Boersma, Y. L., Olinga, P., & Salvati, A. (2020). Time-Resolved Quantification of Nanoparticle Uptake, Distribution, and Impact in Precision-Cut Liver Slices. *Small*, 16(21), 1906523. <https://doi.org/10.1002/smll.201906523>
23. Basu, S., Nagy, J. A., Pal, S., Vasile, E., Eckelhoefer, I. A., Bliss, V. S., Manseau, E. J., Dasgupta, P. S., Dvorak, H. F., & Mukhopadhyay, D. (2001). The neurotransmitter dopamine inhibits angiogenesis induced by vascular permeability factor/vascular endothelial growth factor. *Nature Medicine*, 7(5), 569–574. <https://doi.org/10.1038/87895>
24. Bayraktar, E., Chen, S., Corvigno, S., Liu, J., & Sood, A. K. (2024). Ovarian cancer metastasis: Looking beyond the surface. *Cancer Cell*, 42(10), 1631–1636. <https://doi.org/10.1016/J.CCELL.2024.08.016>
25. Berga, S., & Naftolin, F. (2015). Autonomic nervous regulation of ovarian function by noxious somatic afferent stimulation. *The Journal of Physiological Sciences*, 65(1), 1–9. <https://doi.org/10.3109/09513590.2012.651929>
26. Berrell, N., Kilgallon, A., Donovan, M. L., Lawler, C., Tan, C. W., Embaye, K. S., Tubelleza, R., Monkman, J., Fraser, J. F., O’Byrne, K., Huang, R., & Kulasinghe, A. (2025). Proximity and metabolic activity proxies in the tumor microenvironment as predictors of survival in high grade serous ovarian cancer. *IScience*, 28(10), 113572. <https://doi.org/10.1016/j.isci.2025.113572>
27. Berry, L., Kelly, M., & Miller, L. (2021). Tumor immunogenicity status in high-grade serous ovarian cancer. *Gynecologic Oncology*, 162, S319. [https://doi.org/10.1016/S0090-8258\(21\)01257-9](https://doi.org/10.1016/S0090-8258(21)01257-9)
28. Bokhman, J. V. (1983). Two pathogenetic types of endometrial carcinoma. *Gynecologic Oncology*, 15(1), 10–17. [https://doi.org/10.1016/0090-8258\(83\)90111-7](https://doi.org/10.1016/0090-8258(83)90111-7)
29. Bone, N. B., Liu, Z., Pittet, J.-F., & Zmijewski, J. W. (2017). Frontline Science: D1 dopaminergic receptor signaling activates the AMPK-bioenergetic pathway in macrophages and alveolar epithelial cells and reduces endotoxin-induced ALI. *Journal of Leukocyte Biology*, 101(2), 357–365. <https://doi.org/10.1189/JLB.3HI0216-068RR>
30. Bougherara, H., Mansuet-Lupo, A., Alifano, M., Ngô, C., Damotte, D., Le Frère-Belda, M. A., Donnadieu, E., & Peranzoni, E. (2015). Real-Time Imaging of Resident T Cells in Human Lung and Ovarian Carcinomas Reveals How Different Tumor

- Microenvironments Control T Lymphocyte Migration. *Frontiers in Immunology*, 6(OCT). <https://doi.org/10.3389/FIMMU.2015.00500>
31. Bougherara, H., Némati, F., Nicolas, A., Massonnet, G., Pugnière, M., Ngô, C., Frère-Belda, M.-A. Le, Leary, A., Alexandre, J., Meseure, D., Barret, J.-M., Navarro-Teulon, I., Pèlegri, A., Roman-Roman, S., Prost, J.-F., Donnadieu, E., Decaudin, D., Bougherara, H., Némati, F., ... Decaudin, D. (2017). The humanized anti-human AMHRII mAb 3C23K exerts an anti-tumor activity against human ovarian cancer through tumor-associated macrophages. *Oncotarget*, 8(59), 99950–99965. <https://doi.org/10.18632/oncotarget.21556>
 32. Brasseur, K., Gévry, N., & Asselin, E. (2016). Chemoresistance and targeted therapies in ovarian and endometrial cancers. *Oncotarget*, 8(3), 4008. <https://doi.org/10.18632/ONCOTARGET.14021>
 33. Brauneck, F., Oliveira-Ferrer, L., Muschhammer, J., Sturmheit, T., Ackermann, C., Haag, F., Schulze zur Wiesch, J., Ding, Y., Qi, M., Hell, L., Schmalfeldt, B., Bokemeyer, C., Fiedler, W., & Wellbrock, J. (2023). Immunosuppressive M2 TAMs represent a promising target population to enhance phagocytosis of ovarian cancer cells *in vitro*. *Frontiers in Immunology*, 14, 1250258. <https://doi.org/10.3389/FIMMU.2023.1250258/FULL>
 34. Brodeur, M. N., Simeone, K., Leclerc-Deslauniers, K., Fleury, H., Carmona, E., Provencher, D. M., & Mes-Masson, A. M. (2021). Carboplatin response in preclinical models for ovarian cancer: comparison of 2D monolayers, spheroids, *ex vivo* tumors and *in vivo* models. *Scientific Reports*, 11(1), 18183. <https://doi.org/10.1038/S41598-021-97434-W>
 35. Brückner, L., Reinshagen, A., Hoang, N. A., Höhn, A. K., Lordick, F., Bechmann, I., Aktas, B., Nel, I., & Kallendrusch, S. (2021). FOXM1 Inhibition in Ovarian Cancer Tissue Cultures Affects Individual Treatment Susceptibility *Ex vivo*. *Cancers 2021, Vol. 13, Page 956*, 13(5), 956. <https://doi.org/10.3390/CANCERS13050956>
 36. Burkhard, K. M., Semwal, A., Johnson, B. K., Chu, K. C., Kranick, R. J., Rayan, M., DiFeo, A., Shen, H., & Mehta, G. (2025). Tumoroid model recreates clinically relevant phenotypes of high grade serous ovarian cancer (HGSC) cells, carcinoma associated fibroblasts, and macrophages. *Research Square*, rs.3.rs-6614892. <https://doi.org/10.21203/RS.3.RS-6614892/V1>
 37. Byeon, Y., Lee, J. W., Choi, W. S., Won, J. E., Kim, G. H., Kim, M. G., Wi, T. I., Lee, J. M., Kang, T. H., Jung, I. D., Cho, Y. J., Ahn, H. J., Shin, B. C., Lee, Y. J., Sood, A. K., Han, H. D., & Park, Y. M. (2018). CD44-Targeting PLGA Nanoparticles Incorporating Paclitaxel and FAK siRNA Overcome Chemoresistance in Epithelial Ovarian Cancer. *Cancer Research*, 78(21), 6247–6256. <https://doi.org/10.1158/0008-5472.CAN-17-3871>
 38. Cabarca, S., Ili, C., Vanegas, C., Gil, L., Vertel-Morrison, M., & Brebi, P. (2024). Drug resistance biomarkers in ovarian cancer: a bibliometric study from 2017 to 2022. *Frontiers in Oncology*, 14. <https://doi.org/10.3389/FONC.2024.1450675/FULL>

39. Capellero, S., Erriquez, J., Battistini, C., Porporato, R., Scotto, G., Borella, F., Renzo, M. F. Di, Valabrega, G., & Olivero, M. (2022). *Ovarian Cancer Cells in Ascites Form Aggregates That Display a Hybrid Epithelial-Mesenchymal Phenotype and Allows Survival and Proliferation of Metastasizing Cells*.
<https://doi.org/10.3390/ijms23020833>
40. Charkhchi, P., Cybulski, C., Gronwald, J., Wong, F. O., Narod, S. A., & Akbari, M. R. (2020). CA125 and Ovarian Cancer: A Comprehensive Review. *Cancers*, 12(12), 3730. <https://doi.org/10.3390/CANCERS12123730>
41. Cheasley, D., Nigam, A., Zethoven, M., Hunter, S., Etemadmoghadam, D., Semple, T., Allan, P., Carey, M. S., Fernandez, M. L., Dawson, A., Köbel, M., Huntsman, D. G., Le Page, C., Mes-Masson, A., Provencher, D., Hacker, N., Gao, Y., Bowtell, D., deFazio, A., ... Campbell, I. G. (2021). Genomic analysis of low-grade serous ovarian carcinoma to identify key drivers and therapeutic vulnerabilities. *The Journal of Pathology*, 253(1), 41–54.
<https://doi.org/10.1002/path.5545>
42. Chen, H., Zhou, Y., Tang, Y., Lan, J., Lin, C., Chen, Q., & Kuang, H. (2024). Neutrophil extracellular traps in tumor progression of gynecologic cancers. *Frontiers in Immunology*, 15. <https://doi.org/10.3389/fimmu.2024.1421889>
43. Chen, J., Lin, A., Jiang, A., Qi, C., Liu, Z., Cheng, Q., Yuan, S., & Luo, P. (2025). Computational frameworks transform antagonism to synergy in optimizing combination therapies. *Npj Digital Medicine* 2025 8:1, 8(1), 44-.
<https://doi.org/10.1038/s41746-025-01435-2>
44. Chen, Y., Yan, S. M., Pu, Z., Feng, J., Tan, L., Li, Y., Hu, H., Huang, W., Lin, Y., Peng, Z., He, X., Huang, F., Zhang, H., & Zhang, Y. (2022). Dopamine Signaling Promotes Tissue-Resident Memory Differentiation of CD8+ T Cells and Antitumor Immunity. *Cancer Research*, 82(17), 3130–3142. <https://doi.org/10.1158/0008-5472.CAN-21-4084/706924>
45. Cheng, K. C., Lin, Y. H., Wu, D. S., Shih, I. M., & Wang, T. L. (2025). Macrophages and neutrophils in ovarian cancer microenvironment. *Frontiers in Immunology*, 16, 1677441. <https://doi.org/10.3389/FIMMU.2025.1677441/FULL>
46. Chirshev, E., Hojo, N., Bertucci, A., Sanderman, L., Nguyen, A., Wang, H., Suzuki, T., Brito, E., Martinez, S. R., Castañón, C., Mirshahidi, S., Vazquez, M. E., Wat, P., Oberg, K. C., Ioffe, Y. J., & Untch, J. J. (2020). Epithelial/mesenchymal heterogeneity of high-grade serous ovarian carcinoma samples correlates with miRNA let-7 levels and predicts tumor growth and metastasis. *Molecular Oncology*, 14(11), 2796. <https://doi.org/10.1002/1878-0261.12762>
47. Cho, C. H., Patel, S., & Rajbhandari, P. (2023). Adipose tissue lipid metabolism: lipolysis. *Current Opinion in Genetics & Development*, 83, 102114.
<https://doi.org/10.1016/j.gde.2023.102114>

48. Chocarro-Calvo, A., Zaballos, M. A., Santisteban, P., & García-Jiménez, C. (2012). DARPP-32 Is Required for MAPK/ERK Signaling in Thyroid Cells. *Molecular Endocrinology*, 26(3), 471. <https://doi.org/10.1210/me.2011-1309>
49. Cioffi, M., Dalterio, C., Camerlingo, R., Tirino, V., Consales, C., Riccio, A., Ieranò, C., Cecere, S. C., Losito, N. S., Greggi, S., Pignata, S., Pirozzi, G., & Scala, S. (2015). Identification of a distinct population of CD133+CXCR4+ cancer stem cells in ovarian cancer. *Scientific Reports* 2015 5:1, 5(1), 10357-. <https://doi.org/10.1038/srep10357>
50. Clark, J., Fotopoulou, C., Cunnea, P., & Krell, J. (2022). Novel *Ex vivo* Models of Epithelial Ovarian Cancer: The Future of Biomarker and Therapeutic Research. *Frontiers in Oncology*, 12, 1. <https://doi.org/10.3389/FONC.2022.837233>
51. Coffman, L. G., Pearson, A. T., Frisbie, L. G., Freeman, Z., Christie, E., Bowtell, D. D., & Buckanovich, R. J. (2018). Ovarian Carcinoma-Associated Mesenchymal Stem Cells Arise from Tissue-Specific Normal Stroma. *Stem Cells (Dayton, Ohio)*, 37(2), 257. <https://doi.org/10.1002/STEM.2932>
52. Connor, A. E., Lyons, P. M., Kilgallon, A. M., Simpson, J. C., Perry, A. S., & Lysaght, J. (2024). Examining the evidence for immune checkpoint therapy in high-grade serous ovarian cancer. *Heliyon*, 10(20), e38888. <https://doi.org/10.1016/j.heliyon.2024.e38888>
53. Cortez, A. J., Tudrej, P., Kujawa, K. A., & Lisowska, K. M. (2017). Advances in ovarian cancer therapy. *Cancer Chemotherapy and Pharmacology* 2017 81:1, 81(1), 17–38. <https://doi.org/10.1007/S00280-017-3501-8>
54. Crosbie, E. J., Kitson, S. J., McAlpine, J. N., Mukhopadhyay, A., Powell, M. E., & Singh, N. (2022). Endometrial cancer. *The Lancet*, 399(10333), 1412–1428. [https://doi.org/10.1016/S0140-6736\(22\)00323-3](https://doi.org/10.1016/S0140-6736(22)00323-3)
55. Czerwiński, M., Bednarska-Czerwińska, A., Zmarzły, N., Boroń, D., Oplawski, M., & Grabarek, B. O. (2021). miRNAs in the Expression Regulation of Dopamine-Related Genes and Proteins in Endometrial Cancer. *Journal of Clinical Medicine* 2021, Vol. 10, Page 4939, 10(21), 4939. <https://doi.org/10.3390/JCM10214939>
56. Czogalla, B., Partenheimer, A., Jeschke, U., von Schönfeldt, V., Mayr, D., Mahner, S., Burges, A., Simoni, M., Melli, B., Benevelli, R., Bertini, S., Casarini, L., & Trillsch, F. (2020). β -arrestin 2 Is a Prognostic Factor for Survival of Ovarian Cancer Patients Upregulating Cell Proliferation. *Frontiers in Endocrinology*, 11, 554733. <https://doi.org/10.3389/FENDO.2020.554733/BIBTEX>
57. Dai, Y., Zhao, L., Hua, D., Cui, L., Zhang, X., Kang, N., Qu, L., Li, L., Li, H., Shen, D., Wang, Z., & Wang, J. (2022). Tumor immune microenvironment in endometrial cancer of different molecular subtypes: evidence from a retrospective observational study. *Frontiers in Immunology*, 13, 1035616. <https://doi.org/10.3389/fimmu.2022.1035616>
58. Danhier, F., Ansorena, E., Silva, J. M., Coco, R., Le Breton, A., & Préat, V. (2012). PLGA-based nanoparticles: An overview of biomedical applications. *Journal of Controlled Release*, 161(2), 505–522. <https://doi.org/10.1016/j.jconrel.2012.01.043>

59. Davidson, B., Trope, C. G., & Reich, R. (2014). The Role of the Tumor Stroma in Ovarian Cancer. *Frontiers in Oncology*, 4, 104. <https://doi.org/10.3389/fonc.2014.00104>
60. Davies-Oliveira, J. C., Smith, M. A., Grover, S., Canfell, K., & Crosbie, E. J. (2021). Eliminating Cervical Cancer: Progress and Challenges for High-income Countries. *Clinical Oncology*, 33(9), 550–559. <https://doi.org/10.1016/J.CLON.2021.06.013>
61. Day, C. P., Merlino, G., & Van Dyke, T. (2015). Preclinical Mouse Cancer Models: A Maze of Opportunities and Challenges. *Cell*, 163(1), 39–53. <https://doi.org/10.1016/j.cell.2015.08.068>
62. De Haven Brandon, A., Box, G., Hallsworth, A., Court, W., Matthews, N., Herodek, B., Arteagabeitia, A. B., Valenti, M., & Kirkin, V. (2020). Identification of ovarian high-grade serous carcinoma cell lines that show estrogen-sensitive growth as xenografts in immunocompromised mice. *Scientific Reports* 2020 10:1, 10(1), 10799-. <https://doi.org/10.1038/s41598-020-67533-1>
63. Degos, C., Heinemann, M., Barrou, J., Boucherit, N., Lambaudie, E., Savina, A., Gorvel, L., & Olive, D. (2019). Endometrial tumor microenvironment alters human NK cell recruitment, and resident NK cell phenotype and function. *Frontiers in Immunology*, 10(APR). <https://doi.org/10.3389/fimmu.2019.00877>
64. Deshet-Unger, N., Horn, G., Rawet-Slobodkin, M., Waks, T., Laskov, I., Michaan, N., Raz, Y., Bar, V., Zundeleovich, A., Aharon, S., Turovsky, L., Mallel, G., Salpeter, S., Neev, G., Hollander, K. S., Katz, B. Z., Grisaru, D., & Globerson Levin, A. (2022). Comparing Intraperitoneal and Intravenous Personalized ErbB2CAR-T for the Treatment of Epithelial Ovarian Cancer. *Biomedicines*, 10(9), 2216. <https://doi.org/10.3390/BIOMEDICINES10092216/S1>
65. Dewyse, L., Reynaert, H., & van Grunsven, L. A. (2021). Best practices and progress in precision-cut liver slice cultures. *International Journal of Molecular Sciences*, 22(13), 7137. <https://doi.org/10.3390/IJMS22137137/S1>
66. Dhanush, Y., & Ganesh, V. S. (2025). Non-oncologic to oncologic drug: A systematic review of drug repurposing in cancer. *Cancer Chemotherapy and Pharmacology*, 95(1). <https://doi.org/10.1007/s00280-025-04822-4>
67. Dhillon, A. S., Pollock, C., Steen, H., Shaw, P. E., Mischak, H., & Kolch, W. (2002). Cyclic AMP-Dependent Kinase Regulates Raf-1 Kinase Mainly by Phosphorylation of Serine 259. *Molecular and Cellular Biology*, 22(10), 3237–3246. <https://doi.org/10.1128/mcb.22.10.3237-3246.2002>
68. Domínguez-Ríos, R., Sánchez-Ramírez, D. R., Ruiz-Saray, K., Ocegüera-Basurto, P. E., Almada, M., Juárez, J., Zepeda-Moreno, A., del Toro-Arreola, A., Topete, A., & Daneri-Navarro, A. (2019). Cisplatin-loaded PLGA nanoparticles for HER2 targeted ovarian cancer therapy. *Colloids and Surfaces B: Biointerfaces*, 178, 199–207. <https://doi.org/10.1016/J.COLSURFB.2019.03.011>
69. Dong, M., Böpple, K., Thiel, J., Winkler, B., Liang, C., Schueler, J., Davies, E. J., Barry, S. T., Metsalu, T., Mürdter, T. E., Sauer, G., Ott, G., Schwab, M., & Aulitzky, W. E. (2023). Perfusion Air Culture of Precision-Cut Tumor Slices: An *Ex vivo* System to Evaluate

- Individual Drug Response under Controlled Culture Conditions. *Cells*, 12(5), 807. <https://doi.org/10.3390/CELLS12050807/S1>
70. Dorrigiv, D., Simeone, K., Communal, L., Kendall-Dupont, J., St-Georges-robillard, A., Péant, B., Carmona, E., Mes-Masson, A. M., & Gervais, T. (2021). Microdissected tissue vs tissue slices—a comparative study of tumor explant models cultured on-chip and off-chip. *Cancers*, 13(16). <https://doi.org/10.3390/CANCERS13164208/S1>
 71. Duarte Mendes, A., Freitas, A. R., Vicente, R., Vitorino, M., Vaz Batista, M., Silva, M., & Braga, S. (2023). Adipocyte Microenvironment in Ovarian Cancer: A Critical Contributor? *International Journal of Molecular Sciences* 2023, Vol. 24, Page 16589, 24(23), 16589. <https://doi.org/10.3390/IJMS242316589>
 72. Durante, V., Wittwer, A., Theek, B., Martinez-Osuna, M., Donnadieu, E., Hardt, O., Eckardt, D., Bosio, A., Schallenberg, S., & Herbel, C. (2025). Precision-cut tumor tissue slices, a novel tool to study the tumor microenvironment interactions with chimeric antigen receptor (CAR) T cells. *PLOS One*, 20(8), e0327322. <https://doi.org/10.1371/journal.pone.0327322>
 73. Edwards, M., Caruana, P., Escar, M., & Céspedes, M. V. (2026). Shaping the future one slice at a time: How 3D organotypic tumour slice models are driving drug discovery in ovarian cancer. *Translational Oncology*, 63, 102633. <https://doi.org/10.1016/j.tranon.2025.102633>
 74. El-Hammadi, M. M., & Arias, J. L. (2022). Recent Advances in the Surface Functionalization of PLGA-Based Nanomedicines. *Nanomaterials* 2022, Vol. 12, 12(3). <https://doi.org/10.3390/nano12030354>
 75. Elmahboub, Y., Albash, R., Ahmed, S., & Salah, S. (2025). The Road to Precision Nanomedicine: An Insight on Drug Repurposing and Advances in Nanoformulations for Treatment of Cancer. *AAPS PharmSciTech* 2025 26:8, 26(8), 237-. <https://doi.org/10.1208/s12249-025-03233-1>
 76. Emmanuelli, A., Salvagno, C., Hwang, S. M., Awasthi, D., Sandoval, T. A., Chae, C. S., Cheong, J. G., Tan, C., Iwawaki, T., & Cubillos-Ruiz, J. R. (2024). High-grade serous ovarian cancer development and anti-PD-1 resistance is driven by IRE1 α activity in neutrophils. *Oncoimmunology*, 13(1), 2411070. <https://doi.org/10.1080/2162402X.2024.2411070>
 77. Erciyestepe, M., Dinç Sonuşen, Ş., Seçmeler, Ş., Aydın, O., Öztürk, A. E., Büyükkuşcu, A., Erciyestepe, S. G., Çelik, E., Erturk, K., & Atci, M. M. (2025). Bevacizumab Efficacy and Tolerability in Patients with Metastatic Platinum-Sensitive Ovarian Cancer Beyond Progression. *Cancer Management and Research*, 17, 1679. <https://doi.org/10.2147/CMAR.S531276>
 78. Español, P., Luna, R., Soler, C., Caruana, P., Altés-Arranz, A., Rodríguez, F., Porta, O., Sanchez, O., Llurba, E., Rovira, R., & Céspedes, M. V. (2022). Neural plasticity of the uterus: New targets for endometrial cancer? *Women's Health*, 18, 17455057221095536. <https://doi.org/10.1177/17455057221095537>

79. Español, P., Rovira, R., Caruana, P., Luna-Guibourg, R., Soler, C., Teixeira, N., Rodríguez, F., Gallardo, A., Edwards, M., Porta, O., Gámez, M., Sánchez, O., Llurba, E., Corchero, J. L., & Céspedes, M. V. (2023). Dopamine receptors D1 and D2 show prognostic significance and potential therapeutic applications for endometrial cancer patients. *Gynecologic Oncology*, 176, 25–35. <https://doi.org/10.1016/J.YGYNO.2023.06.019>
80. Estes, J. M., Oliver, P. G., Straughn, J. M., Zhou, T., Wang, W., Grizzle, W. E., Alvarez, R. D., Stockard, C. R., LoBuglio, A. F., & Buchsbaum, D. J. (2007). Efficacy of anti-death receptor 5 (DR5) antibody (TRA-8) against primary human ovarian carcinoma using a novel *ex vivo* tissue slice model. *Gynecologic Oncology*, 105(2), 291–298. <https://doi.org/10.1016/J.YGYNO.2006.12.033>
81. Fader, A. N., & Rose, P. G. (2009). Abraxane for the treatment of gynecologic cancer patients with severe hypersensitivity reactions to paclitaxel. *International Journal of Gynecological Cancer*, 19(7), 1281–1283. <https://doi.org/10.1111/IGC.0b013e3181a38e2f>
82. Fan, T., Lv, Y., Fan, T., & Lv, Y. (2023). The role of DRD2 ubiquitination in macrophage polarization. *European Respiratory Journal*, 62(suppl 67), PA2717. <https://doi.org/10.1183/13993003.congress-2023.pa2717>
83. Fan, Y., Wang, J., Fang, Z., Pierce, S. R., West, L., Staley, A., Tucker, K., Yin, Y., Sun, W., Kong, W., Prabhu, V., Allen, J. E., Zhou, C., & Bae-Jump, V. L. (2022). Anti-Tumor and Anti-Invasive Effects of ONC201 on Ovarian Cancer Cells and a Transgenic Mouse Model of Serous Ovarian Cancer. *Frontiers in Oncology*, 12, 789450. <https://doi.org/10.3389/FONC.2022.789450/FULL>
84. Fang, Z., Wang, J., Clark, L. H., Sun, W., Yin, Y., Kong, W., Pierce, S. R., West, L., Sullivan, S. A., Tran, A.-Q., Prabhu, V. V., Zhou, C., & Bae-Jump, V. (2018). ONC201 demonstrates anti-tumorigenic and anti-metastatic activity in uterine serous carcinoma *in vitro*. *American Journal of Cancer Research*, 8(8), 1551. <https://pmc.ncbi.nlm.nih.gov/articles/PMC6129479/>
85. Fernandez-Garza, L. E., Dominguez-Vigil, I. G., Garza-Martinez, J., Valdez-Aparicio, E. A., Barrera-Barrera, S. A., & Barrera-Saldana, H. A. (2021). Personalized Medicine in Ovarian Cancer: A Perspective From Mexico. *World Journal of Oncology*, 12(4), 85. <https://doi.org/10.14740/wjon1383>
86. Figueroa, C., Gálvez-Cancino, F., Oyarce, C., Contreras, F., Prado, C., Valeria, C., Cruz, S., Lladser, A., & Pacheco, R. (2017). Inhibition of dopamine receptor D3 signaling in dendritic cells increases antigen cross-presentation to CD8+ T-cells favoring anti-tumor immunity. *Journal of Neuroimmunology*, 303, 99–107. <https://doi.org/10.1016/j.jneuroim.2016.12.014>
87. Francoeur, A. A., Ayoub, N., Greenberg, D., & Tewari, K. S. (2025). Drug discovery in advanced and recurrent endometrial cancer: Recent advances. *Oncology Research*, 33(7), 1511. <https://doi.org/10.32604/OR.2025.061120>
88. Frederick, P. J., Kendrick, J. E., Straughn, J. M., Della Manna, D. L., Oliver, P. G., Lin, H. Y., Grizzle, W. E., Stockard, C. R., Alvarez, R. D., Zhou, T., LoBuglio, A. F., & Buchsbaum,

- D. J. (2009). Effect of TRA-8 Anti-Death Receptor 5 Antibody in Combination With Chemotherapy in an *Ex vivo* Human Ovarian Cancer Model. *International Journal of Gynecologic Cancer*, 19(5), 814–819.
<https://doi.org/10.1111/IGC.0B013E3181A2A003>
89. Fu, S., Zhu, X., Huang, F., & Chen, X. (2025). Anti-PEG Antibodies and Their Biological Impact on PEGylated Drugs: Challenges and Strategies for Optimization. *Pharmaceutics*, 17(8), 1074. <https://doi.org/10.3390/pharmaceutics17081074>
 90. Funston, G., O'Flynn, H., Ryan, N. A. J., Hamilton, W., & Crosbie, E. J. (2018). Recognizing Gynecological Cancer in Primary Care: Risk Factors, Red Flags, and Referrals. *Advances in Therapy*, 35(4), 577. <https://doi.org/10.1007/S12325-018-0683-3>
 91. Gabizon, A. A., Gabizon-Peretz, S., Modaresahmadi, S., & La-Beck, N. M. (2025). Thirty years from FDA approval of pegylated liposomal doxorubicin (Doxil/Caelyx): an updated analysis and future perspective. *BMJ Oncology*, 4(1), e000573. <https://doi.org/10.1136/bmjonc-2024-000573>
 92. Garlisi, B., Lauks, S., Aitken, C., Ogilvie, L. M., Lockington, C., Petrik, D., Eichhorn, J. S., & Petrik, J. (2024). The Complex Tumor Microenvironment in Ovarian Cancer: Therapeutic Challenges and Opportunities. *Current Oncology 2024, Vol. 31, Pages 3826–3844*, 31(7), 3826–3844. <https://doi.org/10.3390/curroncol31070283>
 93. Gilham, C., & Peto, J. (2025). Is elimination of cervical cancer in sight in England? *Preventive Medicine*, 191, 108218. <https://doi.org/10.1016/J.YPMED.2025.108218>
 94. Gralewska, P., Gajek, A., Marczak, A., & Rogalska, A. (2024). Targeted Nanocarrier-Based Drug Delivery Strategies for Improving the Therapeutic Efficacy of PARP Inhibitors against Ovarian Cancer. *International Journal of Molecular Sciences*, 25(15), 8304. <https://doi.org/10.3390/IJMS25158304>
 95. Grandy, D. K., Zhang, Y., Bouvier, C., Zhou, Q. Y., Johnson, R. A., Allen, L., Buck, K., Bunzow, J. R., Salon, J., & Civelli, O. (1991). Multiple human D5 dopamine receptor genes: a functional receptor and two pseudogenes. *Proceedings of the National Academy of Sciences of the United States of America*, 88(20), 9175–9179. <https://doi.org/10.1073/PNAS.88.20.9175>
 96. Grant, C. E., Flis, A. L., & Ryan, B. M. (2022). Understanding the role of dopamine in cancer: past, present and future. *Carcinogenesis*, 43(6), 517. <https://doi.org/10.1093/CARCIN/BGAC045>
 97. Gunay, G., Kirit, H. A., Kamatar, A., Baghdasaryan, O., Hamsici, S., & Acar, H. (2020). The effects of size and shape of the ovarian cancer spheroids on the drug resistance and migration. *Gynecologic Oncology*, 159(2), 563–572. <https://doi.org/10.1016/J.YGYNO.2020.09.002>
 98. Guo, L., Chen, D., Yin, X., & Shu, Q. (2019). GSK-3 β Promotes Cell Migration and Inhibits Autophagy by Mediating the AMPK Pathway in Breast Cancer. *Oncology Research*, 27(4), 487. <https://doi.org/10.3727/096504018X15323394008784>

99. Gutiérrez-Martínez, E., Planès, R., Anselmi, G., Reynolds, M., Menezes, S., Adiko, A. C., Saveanu, L., & Guermonprez, P. (2015). Cross-Presentation of Cell-Associated Antigens by MHC Class I in Dendritic Cell Subsets. *Frontiers in Immunology*, 6(JUL), 363. <https://doi.org/10.3389/fimmu.2015.00363>
100. Haak, A. J., Kostallari, E., Sicard, D., Ligresti, G., Choi, K. M., Caporarello, N., Jones, D. L., Tan, Q., Meridew, J., Espinosa, A. M. D., Aravamudhan, A., Maiers, J. L., Britt, R. D., Roden, A. C., Pabelick, C. M., Prakash, Y. S., Nouraie, S. M., Li, X., Zhang, Y., ... Tschumperlin, D. J. (2019). Selective YAP/TAZ inhibition in fibroblasts via dopamine receptor D1 agonism reverses fibrosis. *Science Translational Medicine*, 11(516). <https://doi.org/10.1126/SCITRANSLMED.AAU6296>
101. Halder, S., K Azad, M. A., Shamiul ISLAM, M., Hossen, I., Lata SHUMA, M., & Rahman KABIR, E. (2025). Strategic application of a mixed polymeric micellar solid dispersion system to domperidone for improved biopharmaceutical characteristics. *Journal of Research in Pharmacy*, 27(2), 860–872. <https://doi.org/10.29228/jrp.366>
102. Han, F., Konkalmatt, P., Mokashi, C., Kumar, M., Zhang, Y., Ko, A., Farino, Z. J., Asico, L. D., Xu, G., Gildea, J., Zheng, X., Felder, R. A., Lee, R. E. C., Jose, P. A., Freyberg, Z., & Armando, I. (2019). Dopamine D2 receptor modulates Wnt expression and control of cell proliferation. *Scientific Reports* 2019 9:1, 9(1), 16861-. <https://doi.org/10.1038/s41598-019-52528-4>
103. Han, X., Ni, J., Wu, Z., Wu, J., Li, B., Ye, X., Dai, J., Chen, C., Xue, J., Wan, R., Wen, L., Wang, X., & Hu, G. (2020). Myeloid-specific dopamine D2 receptor signalling controls inflammation in acute pancreatitis via inhibiting M1 macrophage. *British Journal of Pharmacology*, 177(13), 2991. <https://doi.org/10.1111/BPH.15026>
104. He, L., & Deng, C. (2022). Recent advances in organotypic tissue slice cultures for anticancer drug development. *International Journal of Biological Sciences*, 18(15), 5885. <https://doi.org/10.7150/IJBS.78997>
105. Hedrick, C. C., & Malanchi, I. (2021). Neutrophils in cancer: heterogeneous and multifaceted. *Nature Reviews Immunology* 2021 22:3, 22(3), 173–187. <https://doi.org/10.1038/s41577-021-00571-6>
106. Hinshaw, H. D., Smith, A., Rungruang, B., Kelley, J. L., Beriwal, S., Krivak, T. C., Sukumvanich, P., & Olawaiye, A. B. (2013). The Risk of subsequent malignancies in women with uterine papillary serous or clear cell endometrial cancers. *International Journal of Gynecological Cancer*, 23(6), 1044–1049. <https://doi.org/10.1097/IGC.0b013e3182959053>
107. Hollis, R. L., Thomson, J. P., Stanley, B., Churchman, M., Meynert, A. M., Rye, T., Bartos, C., Iida, Y., Croy, I., Mackean, M., Nussey, F., Okamoto, A., Semple, C. A., Gourley, C., & Herrington, C. S. (2020). Molecular stratification of endometrioid ovarian carcinoma predicts clinical outcome. *Nature Communications* 2020 11:1, 11(1), 4995-. <https://doi.org/10.1038/s41467-020-18819-5>
108. Horeweg, N., Workel, H. H., Loiero, D., Church, D. N., Vermij, L., León-Castillo, A., Krog, R. T., de Boer, S. M., Nout, R. A., Powell, M. E., Mileschkin, L. R., MacKay, H., Leary, A.,

- Singh, N., Jürgenliemk-Schulz, I. M., Smit, V. T. H. B. M., Creutzberg, C. L., Koelzer, V. H., Nijman, H. W., ... Bosse, T. (2022a). Tertiary lymphoid structures critical for prognosis in endometrial cancer patients. *Nature Communications* 2022 13:1, 13(1), 1373-. <https://doi.org/10.1038/s41467-022-29040-x>
109. Hu, W., Zhang, L., Ferri-Borgogno, S., Kwan, S. Y., Lewis, K. E., Cun, H. T., Yeung, T. L., Soliman, P. T., Tarapore, R. S., Allen, J. E., Guan, X., Lu, K. H., Mok, S. C., & Au-Yeung, C. L. (2020). Targeting Dopamine Receptor D2 by Imipridone Suppresses Uterine Serous Cancer Malignant Phenotype. *Cancers* 2020, Vol. 12, Page 2436, 12(9), 2436. <https://doi.org/10.3390/CANCERS12092436>
 110. Huang, Y. L., Dickerson, L. K., Kenerson, H., Jiang, X., Pillarisetty, V., Tian, Q., Hood, L., Gujral, T. S., & Yeung, R. S. (2023). Organotypic Models for Functional Drug Testing of Human Cancers. *BME Frontiers*, 4. <https://doi.org/10.34133/BMEF.0022>
 111. Ishida, C. T., Zhang, Y., Bianchetti, E., Shu, C., Nguyen, T. T. T., Kleiner, G., Sanchez-Quintero, M. J., Quinzii, C. M., Westhoff, M. A., Karpel-Massler, G., Prabhu, V. V., Allen, J. E., & Siegelin, M. D. (2018). Metabolic reprogramming by dual AKT/ERK inhibition through imipridones elicits unique vulnerabilities in glioblastoma. *Clinical Cancer Research*, 24(21), 5392–5406. <https://doi.org/10.1158/1078-0432.CCR-18-1040>
 112. Ishizawa, J., Kojima, K., Chachad, D., Ruvolo, P., Ruvolo, V., Jacamo, R. O., Borthakur, G., Mu, H., Zeng, Z., Tabe, Y., Allen, J., Wang, Z., Ma, W., Lee, H., Orlowski, R. Z., Sarbassov, D. D., Neelapu, S. S., McDonnell, T. J., Miranda, R. N., ... Andreeff, M. (2015). ONC201 Induces p53-Independent Apoptosis and Abrogates Stem Cell Function in Hematological Malignancies by Induction of ATF4 through Integrated Stress Response. *Blood*, 126(23), 2051–2051. <https://doi.org/10.1182/blood.v126.23.2051.2051>
 113. Iżycka, N., Zaborowski, M. P., Ciecierski, Ł., Jaz, K., Szubert, S., Miedziarek, C., Rezler, M., Piątek-Bajan, K., Synakiewicz, A., Jankowska, A., Figlerowicz, M., Sterzyńska, K., & Nowak-Markwitz, E. (2023). Cancer Stem Cell Markers—Clinical Relevance and Prognostic Value in High-Grade Serous Ovarian Cancer (HGSOC) Based on The Cancer Genome Atlas Analysis. *International Journal of Molecular Sciences*, 24(16), 12746. <https://doi.org/10.3390/ijms241612746>
 114. Jones, T., Stone, R., Wang, T.-L., Shih, I.-M., & Yeh, Y.-L. (2023). Characterization of myeloid-derived suppressor cells (MDSCs) in endometrial cancer (2292). *Gynecologic Oncology*, 176, S310–S311. <https://doi.org/10.1016/j.ygyno.2023.06.410>
 115. Jones-Tabah, J., Mohammad, H., Paulus, E. G., Clarke, P. B. S., & Hébert, T. E. (2022). The Signaling and Pharmacology of the Dopamine D1 Receptor. *Frontiers in Cellular Neuroscience*, 15, 806618. <https://doi.org/10.3389/FNCEL.2021.806618/FULL>
 116. Joshi, A., & Ellenson, L. H. (2017). PI3K/PTEN/AKT Genetic Mouse Models of Endometrial Carcinoma. *Advances in Experimental Medicine and Biology*, 943, 261–273. https://doi.org/10.1007/978-3-319-43139-0_9
 117. Jumadi, J., Harun, W. S. W., Kadirgama, K., Samylingam, L., Aslfattahi, N., Clint, K. S., Kok, C. K., & Kiai, M. S. (2025). A Comparative Review of Polylactic Acid and Poly(Lactic-

- co-Glycolic Acid) Biomaterials: Optimizing Drug Delivery. *BioNanoScience* 2025 16:1, 16(1), 19-. <https://doi.org/10.1007/s12668-025-02233-6>
118. Kamat, A. A., Merritt, W. M., Coffey, D., Lin, Y. G., Patel, P. R., Broaddus, R., Nugent, E., Han, L. Y., Landen, C. N., Spannuth, W. A., Lu, C., Coleman, R. L., Gershenson, D. M., & Sood, A. K. (2007). Clinical and biological significance of vascular endothelial growth factor in endometrial cancer. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, 13(24), 7487–7495. <https://doi.org/10.1158/1078-0432.CCR-07-1017>
 119. Kang, S. W., Lee, J., Kang, O.-J., Kim, Y.-M., Choi, E. K., & Lee, S.-W. (2023). Transcriptome Profiling and Characterization of Peritoneal Metastasis Ovarian Cancer Xenografts in Humanized Mice. *BioRxiv*, 2023.10.27.563867. <https://doi.org/10.1101/2023.10.27.563867>
 120. Kasikova, L., Rakova, J., Hensler, M., Lanickova, T., Tomankova, J., Pasulka, J., Drozenova, J., Mojzisova, K., Fialova, A., Vosahlikova, S., Laco, J., Ryska, A., Dundr, P., Kocian, R., Brtnicky, T., Skapa, P., Capkova, L., Kovar, M., Prochazka, J., ... Fucikova, J. (2024). Tertiary lymphoid structures and B cells determine clinically relevant T cell phenotypes in ovarian cancer. *Nature Communications* 2024 15:1, 15(1), 2528-. <https://doi.org/10.1038/s41467-024-46873-w>
 121. Kayser, C., Brauer, A., Susanne, S., & Wandmacher, A. M. (2023). The challenge of making the right choice: patient avatars in the era of cancer immunotherapies. *Frontiers in Immunology*, 14, 1237565. <https://doi.org/10.3389/fimmu.2023.1237565>
 122. Kemp, C. J. (2015). Animal models of chemical carcinogenesis: driving breakthroughs in cancer research for 100 years. *Cold Spring Harbor Protocols*, 2015(10), 865. <https://doi.org/10.1101/pdb.top069906>
 123. Khashaba, M., Fawzy, M., Abdel-Aziz, A., Eladawei, G., & Nagib, R. (2022). Subtyping of high grade serous ovarian carcinoma: histopathological and immunohistochemical approach. *Journal of the Egyptian National Cancer Institute*, 34(1), 6. <https://doi.org/10.1186/s43046-022-00104-9>
 124. Kim, J. G., Kim, S. I., Song, S. H., Gu, J. Y., Lee, M., & Kim, H. K. (2022). Diagnostic and prognostic role of circulating neutrophil extracellular trap markers and prekallikrein in patients with high-grade serous ovarian cancer. *Frontiers in Oncology*, 12, 992056. <https://doi.org/10.3389/FONC.2022.992056/BIBTEX>
 125. Kim, Y.-M., & Kahn, M. (2014). The role of the Wnt signaling pathway in cancer stem cells: prospects for drug development. *Research and Reports in Biochemistry*, 4, 1. <https://doi.org/10.2147/rrbc.s53823>
 126. Kirsch-Mangu, A. T., Țîpcu, A., Gâta, V. A., Pop, D. C., Fekete, Z., Irimie, A., & Kubelac, P. M. (2025). Neutrophil to Lymphocyte Ratio a Prognostic Tool in Endometrial Cancer Among Classical Prognostic Factors. *Diagnostics* 2025, Vol. 15, 15(17). <https://doi.org/10.3390/diagnostics15172172>
 127. Kolben, T., Mannewitz, M., Perleberg, C., Schnell, K., Anz, D., Hahn, L., Meister, S., Schmoeckel, E., Burges, A., Czogalla, B., Hester, A., Mahner, S., Kessler, M., Jeschke, U.,

- Corradini, S., Trillsch, F., & Beyer, S. (2022). Presence of regulatory T-cells in endometrial cancer predicts poorer overall survival and promotes progression of tumor cells. *Cellular Oncology (Dordrecht)*, 45(6), 1171. <https://doi.org/10.1007/s13402-022-00708-2>
128. Kopper, O., de Witte, C. J., Löhmußaar, K., Valle-Inclán, J. E., Hami, N., Kester, L., Balgobind, A. V., Korving, J., Proost, N., Begthel, H., van Wijk, L. M., Revilla, S. A., Theeuwssen, R., van de Ven, M., van Roosmalen, M. J., Ponsioen, B., Ho, V. W. H., Neel, B. G., Bosse, T., ... Clevers, H. (2019). An organoid platform for ovarian cancer captures intra- and interpatient heterogeneity. *Nature Medicine* 2019 25:5, 25(5), 838–849. <https://doi.org/10.1038/s41591-019-0422-6>
 129. Korch, C., Spillman, M. A., Jackson, T. A., Jacobsen, B. M., Murphy, S. K., Lessey, B. A., Jordan, V. C., & Bradford, A. P. (2012). DNA profiling analysis of endometrial and ovarian cell lines reveals misidentification, redundancy and contamination. *Gynecologic Oncology*, 127(1), 241. <https://doi.org/10.1016/J.YGYNO.2012.06.017>
 130. Krishna, A., Meynert, A., Dolt, K. S., Kelder, M., Mesropian, A., Ewing, A., Brouwers, C., Claassens, J. W., Linssen, M. M., Sheraz, S., Taylor, G. C., Gautier, P., Ferrer-Vaquer, A., Grimes, G., Becher, H., Silk, R., Gris-Oliver, A., Pinyol, R., Semple, C. A., ... Ozdemir, D. D. (2026). Mutational scanning reveals oncogenic CTNNB1 mutations have diverse effects on signaling. *Nature Genetics* 2026 58:2, 58(2), 366–375. <https://doi.org/10.1038/s41588-025-02496-5>
 131. Kruse, B., Buzzai, A. C., Shridhar, N., Braun, A. D., Gellert, S., Knauth, K., Pozniak, J., Peters, J., Dittmann, P., Mengoni, M., van der Sluis, T. C., Höhn, S., Antoranz, A., Krone, A., Fu, Y., Yu, D., Essand, M., Geffers, R., Mougiakakos, D., ... Tüting, T. (2023). CD4+ T cell-induced inflammatory cell death controls immune-evasive tumours. *Nature* 2023 618:7967, 618(7967), 1033–1040. <https://doi.org/10.1038/s41586-023-06199-x>
 132. Kurman, R. J., & Shih, I. M. (2016). The dualistic model of ovarian carcinogenesis revisited, revised, and expanded. *American Journal of Pathology*, 186(4), 733–747. <https://doi.org/10.1016/j.ajpath.2015.11.011>
 133. Laforêts, F., Kotantaki, P., Malacrida, B., Elorbany, S., Manchanda, R., Donnadieu, E., & Balkwill, F. (2023). Semi-supervised analysis of myeloid and T cell behavior in *ex vivo* ovarian tumor slices reveals changes in cell motility after treatments. *IScience*, 26(4). <https://doi.org/10.1016/J.ISCI.2023.106514>
 134. Lalfer, M., Chappert, P., Carpentier, M., Urbain, D., Davoust, J. M., & Gross, D. A. (2019). Foxp3+ regulatory and conventional CD4+ T cells display similarly high frequencies of alloantigen-reactive cells. *Frontiers in Immunology*, 10(MAR), 432327. <https://doi.org/10.3389/fimmu.2019.00521>
 135. Lee, K. W., Yam, J. W. P., & Mao, X. (2023). Dendritic Cell Vaccines: A Shift from Conventional Approach to New Generations. *Cells*, 12(17), 2147. <https://doi.org/10.3390/cells12172147>
 136. Lheureux, S., N'Diaye, M., Blanc-Fournier, C., Dugué, A. E., Clarisse, B., Dutoit, S., Giffard, F., Abeilard, E., Briand, M., Labiche, A., Grellard, J. M., Crouet, H., Martin, S.,

- Joly, F., & Poulain, L. (2015). Identification of predictive factors of response to the BH3-mimetic molecule ABT-737: An *ex vivo* experiment in human serous ovarian carcinoma. *International Journal of Cancer*, 136(5), E340–E350.
<https://doi.org/10.1002/IJC.29104>
137. Li, E. S., Ho, R., Tao, R., Choi, Y. W. Y., Shin, C. Y., Chen, S. Y., Gokbayrak, B., Senz, J., Yao, B., Johnston, L., Martin, S. D., Yang, E., Carey, M. S., Hennessy, B. T., Huntsman, D. G., Klein Geltink, R. I., Hoang, L., & Wang, Y. (2025). Molecular subtyping of endometrial carcinoma cell lines uncovers subtype-specific targetable vulnerabilities. *Npj Precision Oncology* 9(1): 254-. <https://doi.org/10.1038/s41698-025-01053-x>
 138. Li, H., Sheng, J. J., Zheng, S. A., Liu, P. W., Wu, N., Zeng, W. J., Li, Y. H., & Wang, J. (2025). Platinum-resistant ovarian cancer: From mechanisms to treatment strategies. *Genes & Diseases*, 13(2), 101801. <https://doi.org/10.1016/j.gendis.2025.101801>
 139. Li, X., Peng, X., Zoulikha, M., Bofo, G. F., Magar, K. T., Ju, Y., & He, W. (2024). Multifunctional nanoparticle-mediated combining therapy for human diseases. *Signal Transduction and Targeted Therapy* 2023 9(1): 1 <https://doi.org/10.1038/s41392-023-01668-1>
 140. Li, Z., Li, K., Zhang, C., Zhao, Y., Guo, Y., He, J., Chang, S., Fang, X., Liu, K., Zhu, P., Chen, Z., & Ruan, C. (2025). Bioprinted Organoids: An Innovative Engine in Biomedicine. *Advanced Science*, 12(33), e07317. <https://doi.org/10.1002/ADVS.202507317>
 141. Lim, S. Y., Aboelnasr, L. S., & El-Bahrawy, M. (2025). Tumour-on-Chip Models for the Study of Ovarian Cancer: Current Challenges and Future Prospects. *Cancers*, 17(19), 3239. <https://doi.org/10.3390/CANCERS17193239>
 142. Liu, J. F., Palakurthi, S., Zeng, Q., Zhou, S., Ivanova, E., Huang, W., Zervantonakis, I. K., Selfors, L. M., Shen, Y., Pritchard, C. C., Zheng, M., Adleff, V., Papp, E., Piao, H., Novak, M., Fotheringham, S., Wulf, G. M., English, J., Kirschmeier, P. T., ... Drapkin, R. (2017). Establishment of patient-derived tumor xenograft models of epithelial ovarian cancer for preclinical evaluation of novel therapeutics. *Clinical Cancer Research*, 23(5), 1263–1273. <https://doi.org/10.1158/1078-0432.CCR-16-1237/274377>
 143. Liu, L., Wu, Y., Wang, B., Jiang, Y., Lin, L., Li, X., & Yang, S. (2021a). DA-DRD5 signaling controls colitis by regulating colonic M1/M2 macrophage polarization. *Cell Death & Disease* 2021 12:6, 12(6), 500-. <https://doi.org/10.1038/s41419-021-03778-6>
 144. Liu, Q. F., Feng, Z. Y., Jiang, L. L., Xu, T. T., Li, S. M., & Liu, K. R. (2021b). Immune Cell Infiltration as Signatures for the Diagnosis and Prognosis of Malignant Gynecological Tumors. *Frontiers in Cell and Developmental Biology*, 9, 702451.
<https://doi.org/10.3389/fcell.2021.702451>
 145. Lorusso, D., Ferrandina, G., Colombo, N., Pignata, S., Pietragalla, A., Sonetto, C., Pisano, C., Lapresa, M. T., Savarese, A., Tagliaferri, P., Lombardi, D., Cinieri, S., Breda, E., Sabatucci, I., Sabbatini, R., Conte, C., Cecere, S. C., Maltese, G., & Scambia, G. (2019). Carboplatin-paclitaxel compared to Carboplatin-Paclitaxel-Bevacizumab in advanced or recurrent endometrial cancer: MITO END-2 - A randomized phase II trial. *Gynecologic Oncology*, 155(3), 406–412.

- <https://doi.org/10.1016/j.ygyno.2019.10.013>
146. Lv, L., Zhuang, Y. xin, Zhang, H. wu, Tian, N. nan, Dang, W. zhen, & Wu, S. yu. (2017). Capsaicin-loaded folic acid-conjugated lipid nanoparticles for enhanced therapeutic efficacy in ovarian cancers. *Biomedicine & Pharmacotherapy*, 91, 999–1005. <https://doi.org/10.1016/J.BIOPHA.2017.04.097>
 147. Ma, T. L., Zhou, Y., Zhang, C. Y., Gao, Z. A., & Duan, J. X. (2021). The role and mechanism of β -arrestin2 in signal transduction. *Life Sciences*, 275, 119364. <https://doi.org/10.1016/J.LFS.2021.119364>
 148. Mabuchi, S., & Sasano, T. (2021). Myeloid-Derived Suppressor Cells as Therapeutic Targets in Uterine Cervical and Endometrial Cancers. *Cells*, 10(5), 1073. <https://doi.org/10.3390/cells10051073>
 149. MacFawn, I. P., Magnon, G., Gorecki, G., Kunning, S., Rashid, R., Kaiza, M. E., Atiya, H., Ruffin, A. T., Taylor, S., Soong, T. R., Bao, R., Coffman, L. G., & Bruno, T. C. (2024). The activity of tertiary lymphoid structures in high grade serous ovarian cancer is governed by site, stroma, and cellular interactions. *Cancer Cell*, 42(11), 1864-1881.e5. <https://doi.org/10.1016/j.ccell.2024.09.007>
 150. Makale, M. T., Kesari, S., & Wrasidlo, W. (2017). The autonomic nervous system and cancer. *Biocybernetics and Biomedical Engineering*, 37(3), 443–452. <https://doi.org/10.1016/J.BBE.2017.05.001>
 151. Mancebo, G., Sole-Sedeno, J. M., Pino, O., Miralpeix, E., Mojal, S., Garrigos, L., Lloveras, B., Navarro, P., Gibert, J., Lorenzo, M., Aran, I., Carreras, R., & Alameda, F. (2017). Prognostic impact of CD133 expression in Endometrial Cancer Patients. *Scientific Reports* 2017 7:1, 7(1), 7687-. <https://doi.org/10.1038/s41598-017-08048-0>
 152. Marrella, A., Varani, G., Aiello, M., Vaccari, I., Vitale, C., Mojzisek, M., Degrassi, C., & Scaglione, S. (2021). 3D Fluid-Dynamic Ovarian Cancer Model Resembling Systemic Drug Administration for Efficacy Assay. *Altex*, 38(1), 82–94. <https://doi.org/10.14573/ALTEX.2003131>
 153. Maru, Y., & Hippo, Y. (2019). Current Status of Patient-Derived Ovarian Cancer Models. *Cells*, 8(5), 505. <https://doi.org/10.3390/CELLS8050505>
 154. Mastelic-Gavillet, B., Sarivalasis, A., Lozano, E., Wyss, T., Inoges, S., Jolanda, I., De Vries, M., Dartiguenave, F., Jichlinski, P., Derrè, L., Coukos, G., Melero, I., Harari, A., Romero, P., Viganó, S., & Kandalaft, L. E. (2020). Quantitative and qualitative impairments in dendritic cell subsets of patients with ovarian or prostate cancer. *European Journal of Cancer*, 135, 173–182. <https://doi.org/10.1016/j.ejca.2020.04.036>
 155. Mastelic-Gavillet, B., Sarivalasis, A., Lozano, L. E., Lofek, S., Wyss, T., Melero, I., de Vries, I. J. M., Harari, A., Romero, P., Kandalaft, L. E., & Viganó, S. (2023). Longitudinal analysis of DC subsets in patients with ovarian cancer: Implications for immunotherapy. *Frontiers in Immunology*, 14, 1119371. <https://doi.org/10.3389/FIMMU.2023.1119371/FULL>

156. Matuszewska, K., Pereira, M., Petrik, D., Lawler, J., & Petrik, J. (2021). Normalizing Tumor Vasculature to Reduce Hypoxia, Enhance Perfusion, and Optimize Therapy Uptake. *Cancers*, 13(17), 4444. <https://doi.org/10.3390/cancers13174444>
157. Mazzaglia, C., Sheng, Y., Rodrigues, L. N., Lei, I. M., Shields, J. D., & Huang, Y. Y. S. (2023). Deployable extrusion bioprinting of compartmental tumoroids with cancer associated fibroblasts for immune cell interactions. *Biofabrication*, 15(2), 025005. <https://doi.org/10.1088/1758-5090/ACB1DB>
158. McCabe, A., Zaheed, O., McDade, S. S., & Dean, K. (2023). Investigating the suitability of *in vitro* cell lines as models for the major subtypes of epithelial ovarian cancer. *Frontiers in Cell and Developmental Biology*, 11, 1104514. <https://doi.org/10.3389/FCELL.2023.1104514/BIBTEX>
159. McClung, E. C., & Wenham, R. M. (2016). Profile of bevacizumab in the treatment of platinum-resistant ovarian cancer: current perspectives. *International Journal of Women's Health*, 8, 59. <https://doi.org/10.2147/IJWH.S78101>
160. McKenna, M., Filteau, J. R., Butler, B., Sluis, K., Chungyoun, M., Schimek, N., & Nance, E. (2021). Organotypic whole hemisphere brain slice models to study the effects of donor age and oxygen-glucose-deprivation on the extracellular properties of cortical and striatal tissue. *Journal of Biological Engineering*, 16, 14. <https://doi.org/10.1186/s13036-022-00293-w>
161. Meijer, A., Kruyt, F. A. E., Van Der Zee, A. G. J., Hollema, H., Le, P., Ten Hoor, K. A., Groothuis, G. M. M., Quax, W. J., De Vries, E. G. E., & De Jong, S. (2013). Nutlin-3 preferentially sensitises wild-type p53-expressing cancer cells to DR5-selective TRAIL over rhTRAIL. *British Journal of Cancer*, 109(10), 2685. <https://doi.org/10.1038/BJC.2013.636>
162. Mieulet, V., Garnier, C., Kieffer, Y., Guilbert, T., Nemati, F., Marangoni, E., Renault, G., Chamming's, F., Vincent-Salomon, A., & Mechta-Grigoriou, F. (2021). Stiffness increases with myofibroblast content and collagen density in mesenchymal high grade serous ovarian cancer. *Scientific Reports*, 11(1), 4219. <https://doi.org/10.1038/S41598-021-83685-0>
163. Mikulak, J., Terzoli, S., Marzano, P., Cazzetta, V., Martiniello, G., Piazza, R., Viano, M. E., Vitobello, D., Portuesi, R., Grizzi, F., Hegazi, M. A. A., Fiamengo, B., Basso, G., Parachini, L., Mannarino, L., D'Incalci, M., Marchini, S., & Mavilio, D. (2025). Immune evasion mechanisms in early-stage I high-grade serous ovarian carcinoma: insights into regulatory T cell dynamics. *Cell Death & Disease* 2025 16:1, 16(1), 229-. <https://doi.org/10.1038/s41419-025-07557-5>
164. Mirabelli, P., Coppola, L., & Salvatore, M. (2019). Cancer Cell Lines Are Useful Model Systems for Medical Research. *Cancers*, 11(8), 1098. <https://doi.org/10.3390/CANCERS11081098>
165. Miserocchi, G., Mercatali, L., Liverani, C., De Vita, A., Spadazzi, C., Pieri, F., Bongiovanni, A., Recine, F., Amadori, D., & Ibrahim, T. (2017). Management and potentialities of primary cancer cultures in preclinical and translational studies.

- Journal of Translational Medicine*, 15(1), 229. <https://doi.org/10.1186/S12967-017-1328-Z>
166. Misra, S., Moro, C. F., Del Chiaro, M., Pouso, S., Sebestyén, A., Löhr, M., Björnstedt, M., & Verbeke, C. S. (2019). *Ex vivo* organotypic culture system of precision-cut slices of human pancreatic ductal adenocarcinoma. *Scientific Reports* 2019 9:1, 9(1), 2133-. <https://doi.org/10.1038/s41598-019-38603-w>
 167. Mohi-ud-din, R., Chawla, A., Sharma, P., Mir, P. A., Potoo, F. H., Reiner, Ž., Reiner, I., Ateşşahin, D. A., Sharifi-Rad, J., Mir, R. H., & Calina, D. (2023). Repurposing approved non-oncology drugs for cancer therapy: a comprehensive review of mechanisms, efficacy, and clinical prospects. *European Journal of Medical Research* 2023 28:1, 28(1), 345-. <https://doi.org/10.1186/s40001-023-01275-4>
 168. Monneret, C. (2011). Platinum anticancer drugs. From serendipity to rational design. *Annales Pharmaceutiques Francaises*, 69(6), 286–295. <https://doi.org/10.1016/j.pharma.2011.10.001>
 169. Moreno-Smith, M., Lee, S. J., Chunhua, L., Nagaraja, A. S., He, G., Rupaimoole, R., Han, H. D., Jennings, N. B., Roh, J. W., Nishimura, M., Kang, Y., Allen, J. K., Armaiz, G. N., Matsuo, K., Shahzad, M. M. K., Bottsford-Miller, J., Langley, R. R., Cole, S. W., Lutgendorf, S. K., ... Sood, A. K. (2013). Biologic Effects of Dopamine on Tumor Vasculature in Ovarian Carcinoma. *Neoplasia (New York, N.Y.)*, 15(5), 502. <https://doi.org/10.1593/NEO.121412>
 170. Mukherjee, A., Chiang, C. Y., Daifotis, H. A., Nieman, K. M., Fahrman, J. F., Lastra, R. R., Romero, I. L., Fiehn, O., & Lengyel, E. (2020). Adipocyte-induced FABP4 expression in ovarian cancer cells promotes metastasis and mediates carboplatin resistance. *Cancer Research*, 80(8), 1748–1761. <https://doi.org/10.1158/0008-5472.CAN-19-1999/653913/AM/ADIPOCYTE-INDUCED-FABP4-EXPRESSION-IN-OVARIAN>
 171. Mun, S., Lee, H. J., & Kim, P. (2024). Rebuilding the microenvironment of primary tumors in humans: a focus on stroma. *Experimental & Molecular Medicine* 2024 56:3, 56(3), 527–548. <https://doi.org/10.1038/s12276-024-01191-5>
 172. Murali, R., Soslow, R. A., & Weigelt, B. (2014). Classification of endometrial carcinoma: More than two types. *The Lancet Oncology*, 15(7). [https://doi.org/10.1016/S1470-2045\(13\)70591-6](https://doi.org/10.1016/S1470-2045(13)70591-6)
 173. Nagamine, M., Ogi, H., Hirai, M., Omatsu, I., Moriwaki, S., Shibata, S., Yasukawa, S., Yoriki, K., Kojima, M., Mori, T., Itoh, K., & Konishi, E. (2025). Low density of intratumoral M1-macrophage infiltration may correlate with worse prognosis in low-grade early-stage uterine endometrioid carcinoma. *Gynecologic Oncology Reports*, 59(5), 101758. <https://doi.org/10.1016/j.gore.2025.101758>
 174. Naipal, K. A. T., Verkaik, N. S., Sánchez, H., van Deurzen, C. H. M., den Bakker, M. A., Hoeijmakers, J. H. J., Kanaar, R., Vreeswijk, M. P. G., Jager, A., & van Gent, D. C. (2016). Tumor slice culture system to assess drug response of primary breast cancer. *BMC Cancer* 2016 16:1, 16(1), 78-. <https://doi.org/10.1186/s12885-016-2119-2>

175. Nolan, R. A., Muir, R., Runner, K., Haddad, E. K., & Gaskill, P. J. (2018). Role of macrophage dopamine receptors in mediating cytokine production: Implications for neuroinflammation in the context of HIV-associated neurocognitive disorders. *Journal of Neuroimmune Pharmacology : The Official Journal of the Society on NeuroImmune Pharmacology*, 14(1), 134. <https://doi.org/10.1007/S11481-018-9825-2>
176. Nürnberg, B., & Beer-Hammer, S. (2019). Function, Regulation and Biological Roles of PI3Kγ Variants. *Biomolecules*, 9(9), 427. <https://doi.org/10.3390/BIOM9090427>
177. O'Brien, E., Mayer, C. M., & Arend, R. C. (2025). Exploring T-cell bispecific antibodies in gynecologic malignancy. *Gynecologic Oncology Reports*, 59(14), 101772. <https://doi.org/10.1016/j.gore.2025.101772>
178. Okla, K. (2023). Myeloid-Derived Suppressor Cells (MDSCs) in Ovarian Cancer—Looking Back and Forward. *Cells*, 12(14), 1912. <https://doi.org/10.3390/CELLS12141912>
179. Onstad, M. A., Schmandt, R. E., & Lu, K. H. (2016). Addressing the Role of Obesity in Endometrial Cancer Risk, Prevention, and Treatment. *Journal of Clinical Oncology*, 34(35), 4225. <https://doi.org/10.1200/JCO.2016.69.4638>
180. Panyavaranant, P., Teerapakpinyo, C., Pohthipornthawat, N., Oranratanaphan, S., Shuangshoti, S., & Triratanachai, S. (2019). RAS Mutation in Mucinous Carcinoma of the Ovary. *Asian Pacific Journal of Cancer Prevention*, 20(4), 1127–1132. <https://doi.org/10.31557/APJCP.2019.20.4.1127>
181. Parker, J. J., Lizarraga, M., Waziri, A., & Foshay, K. M. (2017). A Human Glioblastoma Organotypic Slice Culture Model for Study of Tumor Cell Migration and Patient-specific Effects of Anti-Invasive Drugs. *Journal of Visualized Experiments : JoVE*, 2017(125), 53557. <https://doi.org/10.3791/53557>
182. Peralta-Cuevas, E., Degollado-Hernández, N. Y., Martínez-Ortiz, I. C., Gutierrez-Onofre, A. J., Garcia-Atutxa, I., & Villanueva-Flores, F. (2026). How do nanoparticle properties shape pharmacokinetics and pharmacodynamics? A mechanistic review. *Frontiers in Pharmacology*, 16, 1704814. <https://doi.org/10.3389/fphar.2025.1704814>
183. Peters, M. A. M., Meijer, C., Fehrmann, R. S. N., Walenkamp, A. M. E., Kema, I. P., de Vries, E. G. E., Hollema, H., & Oosting, S. F. (2019). Serotonin and Dopamine Receptor Expression in Solid Tumours Including Rare Cancers. *Pathology & Oncology Research* 2019 26:3, 26(3), 1539–1547. <https://doi.org/10.1007/S12253-019-00734-W>
184. Pierce, S. R., Fang, Z., Yin, Y., West, L., Asher, M., Hao, T., Zhang, X., Tucker, K., Staley, A., Fan, Y., Sun, W., Moore, D. T., Xu, C., Tsai, Y. H., Parker, J., Prabhu, V. V., Allen, J. E., Lee, D., Zhou, C., & Bae-Jump, V. (2021). Targeting dopamine receptor D2 as a novel therapeutic strategy in endometrial cancer. *Journal of Experimental and Clinical Cancer Research*, 40(1). <https://doi.org/10.1186/S13046-021-01842-9>
185. Pillay, J., Den Braber, I., Vrisekoop, N., Kwast, L. M., De Boer, R. J., Borghans, J. A. M., Tesselaar, K., & Koenderman, L. (2010). *In vivo* labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. *Blood*, 116(4), 625–627. <https://doi.org/10.1182/BLOOD-2010-01-259028>

186. Piulats, J. M., Guerra, E., Gil-Martín, M., Roman-Canal, B., Gatiús, S., Sanz-Pamplona, R., Velasco, A., Vidal, A., & Matias-Guiu, X. (2017). Molecular approaches for classifying endometrial carcinoma. *Gynecologic Oncology*, 145(1), 200–207. <https://doi.org/10.1016/j.ygyno.2016.12.015>
187. Prasad, V., De Jesús, K., & Mailankody, S. (2017). The high price of anticancer drugs: origins, implications, barriers, solutions. *Nature Reviews Clinical Oncology* 2017 14:6, 14(6), 381–390. <https://doi.org/10.1038/nrclinonc.2017.31>
188. Qin, T., Fan, J., Lu, F., Zhang, L., Liu, C., Xiong, Q., Zhao, Y., Chen, G., & Sun, C. (2022). Harnessing preclinical models for the interrogation of ovarian cancer. *Journal of Experimental & Clinical Cancer Research : CR*, 41(1), 277. <https://doi.org/10.1186/S13046-022-02486-Z>
189. Qin, T., Wang, C., Chen, X., Duan, C., Zhang, X., Zhang, J., Chai, H., Tang, T., Chen, H., Yue, J., Li, Y., & Yang, J. (2015). Dopamine induces growth inhibition and vascular normalization through reprogramming M2-polarized macrophages in rat C6 glioma. *Toxicology and Applied Pharmacology*, 286(2), 112–123. <https://doi.org/10.1016/J.TAAP.2015.03.021>
190. Qing, J., Ren, Y., Zhang, Y., Yan, M., Zhang, H., Wu, D., Ma, Y., Chen, Y., Huang, X., Wu, Q., Mazhar, M., Wang, L., Liu, J., Ding, B. Sen, & Cao, Z. (2022). Dopamine receptor D2 antagonism normalizes profibrotic macrophage-endothelial crosstalk in non-alcoholic steatohepatitis. *Journal of Hepatology*, 76(2), 394–406. <https://doi.org/10.1016/j.jhep.2021.09.032>
191. Quevedo, R., Smirnov, P., Tkachuk, D., Ho, C., El-Hachem, N., Safikhani, Z., Pugh, T. J., & Haibe-Kains, B. (2020). Assessment of Genetic Drift in Large Pharmacogenomic Studies. *Cell Systems*, 11(4), 393–401.e2. <https://doi.org/10.1016/J.CELS.2020.08.012>
192. Radu, C. A., Matos de Melo Fernandes, N., Khalfé, S., & Stordal, B. (2023). Awareness of ovarian cancer symptoms and risk factors in a young ethnically diverse British population. *Cancer Medicine*, 12(8), 9879–9892. <https://doi.org/10.1002/CAM4.5670>
193. Rahbani, J.F., Scholtes, C., Lagarde, D.M., Hussain, M.F., Roesler, A., Dykstra, C.B., Bunk, J., et al. (2022). ADRA1A–Gαq signalling potentiates adipocyte thermogenesis through CKB and TNAP. *Nature Metabolism* 4, 1459–1473. <https://doi.org/10.1038/s42255-022-00667-w>
194. Rask, K., Nilsson, A., Brännström, M., Carlsson, P., Hellberg, P., Janson, P. O., Hedin, L., & Sundfeldt, K. (2003). Wnt-signalling pathway in ovarian epithelial tumours: increased expression of β-catenin and GSK3β. *British Journal of Cancer*, 89(7), 1298. <https://doi.org/10.1038/sj.bjc.6601265>
195. Ray, A., Fornsgaglio, J., Dogan, S., Hedau, S., Naik, S. D., & De, A. (2018). Gynaecological cancers and leptin: A focus on the endometrium and ovary. *Facts, Views & Vision in ObGyn*, 10(1), 5. <https://pmc.ncbi.nlm.nih.gov/articles/PMC6260667/>
196. Rivera-Maya, O. B., Ortiz-Robles, C. D., Palacios-Valladares, J. R., & Calderón-Aranda, E. S. (2024). Dopamine D1-Like Receptor Stimulation Induces CREB, Arc, and BDNF

- Dynamic Changes in Differentiated SH-SY5Y Cells. *Neurochemical Research*, 50(1), 35. <https://doi.org/10.1007/S11064-024-04293-8>
197. Rondou, P., Haegeman, G., & Van Craenenbroeck, K. (2010). The dopamine D4 receptor: biochemical and signalling properties. *Cellular and Molecular Life Sciences: CMLS*, 67(12), 1971. <https://doi.org/10.1007/S00018-010-0293-Y>
 198. Rosas-Cruz, A., Salinas-Jazmín, N., & Velázquez, M. A. V. (2021). Dopamine Receptors in Cancer: Are They Valid Therapeutic Targets? *Technology in Cancer Research and Treatment*, 20. <https://doi.org/10.1177/15330338211027913>
 199. Ross, L., Carey, K., & Koenigsberg, R. (2025). Central Nervous System Metastases in Endometrial Carcinoma: A Case Series. *Cureus*, 17(3), e80464. <https://doi.org/10.7759/cureus.80464>
 200. Rubinstein, M. M., Dickinson, S., Narayan, P., Zhou, Q., Iasonos, A., Ma, W., Lakhman, Y., & Makker, V. (2021). Bevacizumab in advanced endometrial cancer. *Gynecologic Oncology*, 161(3), 720–726. <https://doi.org/10.1016/j.ygyno.2021.04.016>
 201. Ruscito, I., Cacsire Castillo-Tong, D., Vergote, I., Ignat, I., Stanske, M., Vanderstichele, A., Ganapathi, R. N., Glajzer, J., Kulbe, H., Trillsch, F., Mustea, A., Kreuzinger, C., Benedetti Panici, P., Gourley, C., Gabra, H., Kessler, M., Sehouli, J., Darb-Esfahani, S., & Braicu, E. I. (2017). Exploring the clonal evolution of CD133/aldehyde-dehydrogenase-1 (ALDH1)-positive cancer stem-like cells from primary to recurrent high-grade serous ovarian cancer (HGSOC). A study of the Ovarian Cancer Therapy–Innovative Models Prolong Survival (OCTIPS).... *European Journal of Cancer*, 79, 214–225. <https://doi.org/10.1016/j.ejca.2017.04.016>
 202. Santi, A., Kugeratski, F. G., & Zanivan, S. (2018). Cancer Associated Fibroblasts: The Architects of Stroma Remodeling. *Proteomics*, 18(5–6), 1700167. <https://doi.org/10.1002/pmic.201700167>
 203. Santo, V. E., Rebelo, S. P., Estrada, M. F., Alves, P. M., Boghaert, E., & Brito, C. (2017). Drug screening in 3D *in vitro* tumor models: overcoming current pitfalls of efficacy read-outs. *Biotechnology Journal*, 12(1), 1600505. <https://doi.org/10.1002/biot.201600505>
 204. Sarkar, C., Chakroborty, D., Goswami, S., Fan, H., Mo, X., & Basu, S. (2022). VEGF-A controls the expression of its regulator of angiogenic functions, dopamine D2 receptor, on endothelial cells. *Journal of Cell Science*, 135(11), jcs259617. <https://doi.org/10.1242/jcs.259617>
 205. Satora, M., Kułak, K., Zaremba, B., Grunwald, A., Świechowska-Starek, P., & Tarkowski, R. (2024). New hopes and promises in the treatment of ovarian cancer focusing on targeted treatment—a narrative review. *Frontiers in Pharmacology*, 15, 1416555. <https://doi.org/10.3389/FPHAR.2024.1416555/BIBTEX>
 206. Secord, A. A., Powell, M. A., & McAlpine, J. (2025). Molecular Characterization and Clinical Implications of Endometrial Cancer. *Obstetrics and Gynecology*, 146(5), 660–671. <https://doi.org/10.1097/AOG.0000000000006080>

207. Seyhan, A. A. (2019). Lost in translation: the valley of death across preclinical and clinical divide – identification of problems and overcoming obstacles. *Translational Medicine Communications* 2019 4:1, 4(1), 1–19. <https://doi.org/10.1186/S41231-019-0050-7>
208. Shan, Y., Pang, M., Wang, L., Mao, Y., Yan, R., Zhou, C., Ji, J., Mao, Y., Jin, Y., & Yang, H. (2024). Increased stiffness of extracellular matrix enhanced chemoresistance in 3D-bioprinted ovarian cancer model. *International Journal of Bioprinting* 2024, 10(3), 1673, 10(3), 1673. <https://doi.org/10.36922/IJB.1673>
209. Shao, X., Zhao, X., Wang, B., Fan, J., Wang, J., & An, H. (2025). Tumor microenvironment targeted nano-drug delivery systems for multidrug resistant tumor therapy. *Theranostics*, 15(5), 1689. <https://doi.org/10.7150/thno.103636>
210. Sim, S. J., Jang, J. H., Choi, J. S., & Chun, K. S. (2024). Domperidone, a Dopamine Receptor D2 Antagonist, Induces Apoptosis by Inhibiting the ERK/STAT3-Mediated Pathway in Human Colon Cancer HCT116 Cells. *Biomolecules & Therapeutics*, 32(5), 568–576. <https://doi.org/10.4062/biomolther.2024.048>
211. Simpkins, F., Drake, R., Escobar, P. F., Nutter, B., Rasool, N., & Rose, P. G. (2015). A phase II trial of paclitaxel, carboplatin, and bevacizumab in advanced and recurrent endometrial carcinoma (EMCA). *Gynecologic Oncology*, 136(2), 240–245. <https://doi.org/10.1016/j.ygyno.2014.12.004>
212. Sivakumar, R., Chan, M., Shin, J. S., Nishida-Aoki, N., Kenerson, H. L., Elemento, O., Beltran, H., Yeung, R., & Gujral, T. S. (2019). Organotypic tumor slice cultures provide a versatile platform for immuno-oncology and drug discovery. *Oncolimmunology*, 8(12). <https://doi.org/10.1080/2162402X.2019.1670019>
213. Sobczuk, P., Łomiak, M., & Cudnoch-Jędrzejewska, A. (2020). Dopamine D1 Receptor in Cancer. *Cancers* 2020, Vol. 12, 12(11), 1–22. <https://doi.org/10.3390/cancers12113232>
214. Sookhai, S., Wang, J. H., McCourt, M., O'Connell, D., & Redmond, H. P. (1999). Dopamine induces neutrophil apoptosis through a dopamine D-1 receptor-independent mechanism. *Surgery*, 126(2), 314–322. [https://doi.org/10.1016/S0039-6060\(99\)70171-6](https://doi.org/10.1016/S0039-6060(99)70171-6)
215. Spinelli, L., D'Anna, P., Morretta, E., Cassiano, C., Piccolo, V., De Rosa, M., Amico, R., De Cicco, P., Brancaccio, D., Conte, C., Zampella, A., Quaglia, F., & Monti, M. C. (2025). PEGylation-Driven Remodeling of the Protein Corona on PLGA Nanoparticles: Implications for Macrophage Recognition. *Biomacromolecules*, 26(12), 8522–8534. <https://doi.org/10.1021/acs.biomac.5c01369>
216. Steinkamp, M. P., Lagutina, I., Brayer, K. J., Schultz, F., Burke, D., Pankratz, V. S., Adams, S. F., Hudson, L. G., Ness, S. A., & Wandinger-Ness, A. (2023). Humanized Patient-derived Xenograft Models of Disseminated Ovarian Cancer Recapitulate Key Aspects of the Tumor Immune Environment within the Peritoneal Cavity. *Cancer Research Communications*, 3(2), 309. <https://doi.org/10.1158/2767-9764.CRC-22-0300>

217. Stelloo, E., Nout, R. A., Osse, E. M., Jürgenliemk-Schulz, I. J., Jobsen, J. J., Lutgens, L. C., Van Der Steen-Banasik, E. M., Nijman, H. W., Putter, H., Bosse, T., Creutzberg, C. L., & Smit, V. T. H. B. M. (2016). Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-stage Endometrial Cancer-Combined Analysis of the PORTEC Cohorts. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, 22(16), 4215–4224. <https://doi.org/10.1158/1078-0432.CCR-15-2878>
218. Stylianopoulos, T., Munn, L. L., & Jain, R. K. (2018). Reengineering the Tumor Vasculature: Improving Drug Delivery and Efficacy. *Trends in Cancer*, 4(4), 258. <https://doi.org/10.1016/j.trecan.2018.02.010>
219. Subramaniam, K. S., Tham, S. T., Mohamed, Z., Woo, Y. L., Mat Adenan, N. A., & Chung, I. (2013). Cancer-Associated Fibroblasts Promote Proliferation of Endometrial Cancer Cells. *PLOS ONE*, 8(7), e68923. <https://doi.org/10.1371/journal.pone.0068923>
220. Sun, R., Zhao, Y., Liu, Y., Zhang, M., Qiu, Z., Ma, X., Wei, L., Lu, W., Liu, Z., & Jiang, J. (2025). Extracellular matrix stiffness in endometrial cancer: driving progression and modulating treatment sensitivity via the ROCK1/YAP1 axis. *Cell Death & Disease*, 16(1). <https://doi.org/10.1038/s41419-025-07697-8>
221. Sun, T., Rodriguez, M., & Kim, L. (2009). Glycogen synthase kinase 3 in the world of cell migration. *Development Growth and Differentiation*, 51(9), 735–742. <https://doi.org/10.1111/j.1440-169X.2009.01141.x>
222. Sun, X. X., Nosrati, Z., Ko, J., Lee, C. M., Bennewith, K. L., & Bally, M. B. (2023). Induced Vascular Normalization—Can One Force Tumors to Surrender to a Better Microenvironment? *Pharmaceutics* 2023, Vol. 15, 15(8). <https://doi.org/10.3390/pharmaceutics15082022>
223. Sun, X., Zhao, R., Zhao, E., Wang, Q., Lian, W., & Xiong, J. (2022). Targeting CD44-positive ovarian cancers via engineered paclitaxel prodrug nanoparticles for enhanced chemotherapeutic efficacy. *Biomedicine & Pharmacotherapy*, 154, 113655. <https://doi.org/10.1016/J.BIOPHA.2022.113655>
224. Suster, N. K., & Virant-Klun, I. (2019). Presence and role of stem cells in ovarian cancer. *World Journal of Stem Cells*, 11(7), 383–397. <https://doi.org/10.4252/wjsc.v11.i7.383>
225. Tamura, K., Miyato, H., Kanamaru, R., Sadatomo, A., Takahashi, K., Ohzawa, H., Koyanagi, T., Saga, Y., Takei, Y., Fujiwara, H., Lefor, A. K., Sata, N., & Kitayama, J. (2022). Neutrophil extracellular traps (NETs) reduce the diffusion of doxorubicin which may attenuate its ability to induce apoptosis of ovarian cancer cells. *Heliyon*, 8(6). <https://doi.org/10.1016/j.heliyon.2022.e09730>
226. Tan, B., Gan, S., Wang, X., Liu, W., & Li, X. (2021). Applications of 3D bioprinting in tissue engineering: advantages, deficiencies, improvements, and future perspectives. *Journal of Materials Chemistry B*, 9(27), 5385–5413. <https://doi.org/10.1039/D1TB00172H>

227. Tang, D., Zhang, S., Shi, X., Wu, J., Yin, G., Tan, X., Liu, F., Wu, X., & Du, X. (2019). Combination of Astragali Polysaccharide and Curcumin Improves the Morphological Structure of Tumor Vessels and Induces Tumor Vascular Normalization to Inhibit the Growth of Hepatocellular Carcinoma. *Integrative Cancer Therapies*, 18, 1534735418824408. <https://doi.org/10.1177/1534735418824408>
228. Tang, H., Chen, H., Jia, Y., Liu, X., Hhan, Z., Wang, A., Liu, Q., Li, X., & Feng, X. (2017). Effect of inhibitors of endocytosis and NF- κ B signal pathway on folate-conjugated nanoparticle endocytosis by rat Kupffer cells. *International Journal of Nanomedicine*, 12, 6937–6947. <https://doi.org/10.2147/IJN.S141407>
229. Tapponi, S., Yusuf, A., Alsaafin, F., & Hussain, Z. (2025). Breaking barriers with pH-responsive nanocarriers: a new frontier in precision oncology. *International Journal of Pharmaceutics*, 682, 125931. <https://doi.org/10.1016/j.ijpharm.2025.125931>
230. Tavares, V., Marques, I. S., Melo, I. G. de, Assis, J., Pereira, D., & Medeiros, R. (2024). Paradigm Shift: A Comprehensive Review of Ovarian Cancer Management in an Era of Advancements. *International Journal of Molecular Sciences* 2024, Vol. 25, Page 1845, 25(3), 1845. <https://doi.org/10.3390/IJMS25031845>
231. Teng, F., Tian, W. Y., Wang, Y. M., Zhang, Y. F., Guo, F., Zhao, J., Gao, C., & Xue, F. X. (2016). Cancer-associated fibroblasts promote the progression of endometrial cancer via the SDF-1/CXCR4 axis. *Journal of Hematology & Oncology*, 9(1), 8. <https://doi.org/10.1186/s13045-015-0231-4>
232. Teng, X., Chen, S., Wang, Q., Chen, Z., Wang, X., Huang, N., & Zheng, S. (2022). Structural insights into G protein activation by D1 dopamine receptor. *Science Advances*, 8(23), eabo4158. <https://doi.org/10.1126/SCIADV.ABO4158>
233. Thatipamula, R. P., Palem, C. R., Gannu, R., Mudragada, S., & Yamsani, M. R. (2011). Formulation and *in vitro* characterization of domperidone loaded solid lipid nanoparticles and nanostructured lipid carriers. *DARU: Journal of Faculty of Pharmacy, Tehran University of Medical Sciences*, 19(1), 23. <https://pmc.ncbi.nlm.nih.gov/articles/PMC3232070/>
234. Tillmanns, T., Masri, A., Stewart, C., Chase, D., Karnezis, A., Chen, L. may, & Urban, R. (2024). Advanced endometrial cancer—The next generation of treatment: A society of gynecologic oncology journal club clinical commentary. *Gynecologic Oncology Reports*, 55, 101462. <https://doi.org/10.1016/J.GORE.2024.101462>
235. Tomás-Pérez, S., Oto, J., Aghababayan, C., Herranz, R., Cuadros-Lozano, A., González-Cantó, E., Mc Cormack, B., Arrés, J., Castaño, M., Cana, F., Martínez-Fernández, L., Santonja, N., Ramírez, R., Herreros-Pomares, A., Cañete-Mota, S., Lluca, A., Marí-Alexandre, J., Medina, P., & Gilabert-Estellés, J. (2023). Increased levels of NETosis biomarkers in high-grade serous ovarian cancer patients' biofluids: Potential role in disease diagnosis and management. *Frontiers in Immunology*, 14, 1111344. <https://doi.org/10.3389/>

236. Tong, F., Wang, Y., & Gao, H. (2024). Progress and challenges in the translation of cancer nanomedicines. *Current Opinion in Biotechnology*, 85, 103045. <https://doi.org/10.1016/j.copbio.2023.103045>
237. Tong, X., Liu, Y., Xu, X., Shi, J., Hu, W., Ma, T., Cui, P., Lu, W., Pei, Z., Xu, M., Zhang, F., Li, X., & Feng, Y. (2020). Ovarian Innervation Coupling With Vascularity: The Role of Electro-Acupuncture in Follicular Maturation in a Rat Model of Polycystic Ovary Syndrome. *Frontiers in Physiology*, 11, 526285. <https://doi.org/10.3389/FPHYS.2020.00474/BIBTEX>
238. Tsankof, A., & Tziomalos, K. (2022). Adiponectin: A player in the pathogenesis of hormone-dependent cancers. *Frontiers in Endocrinology*, 13, 1018515. <https://doi.org/10.3389/FENDO.2022.1018515/BIBTEX>
239. Tu, Y. sheng, He, J., Liu, H., Lee, H. C., Wang, H., Ishizawa, J., Allen, J. E., Andreeff, M., Orlowski, R. Z., Davis, R. E., & Yang, J. (2017). The Imipridone ONC201 Induces Apoptosis and Overcomes Chemotherapy Resistance by Up-Regulation of Bim in Multiple Myeloma. *Neoplasia (United States)*, 19(10), 772–780. <https://doi.org/10.1016/j.neo.2017.07.009>
240. Tucker, K., Yin, Y., Staley, S.-A., Zhao, Z., Fang, Z., Fan, Y., Zhang, X., Suo, H., Sun, W., Prabhu, V. V., Allen, J. E., Zhou, C., & Bae-Jump, V. L. (2022). ONC206 has anti-tumorigenic effects in human ovarian cancer cells and in a transgenic mouse model of high-grade serous ovarian cancer. *American Journal of Cancer Research*, 12(2), 521. <https://pmc.ncbi.nlm.nih.gov/articles/PMC8900003/>
241. Tung, M. C., Lin, Y. W., Lee, W. J., Wen, Y. C., Liu, Y. C., Chen, J. Q., Hsiao, M., Yang, Y. C., & Chien, M. H. (2022). Targeting DRD2 by the antipsychotic drug, penfluridol, retards growth of renal cell carcinoma via inducing stemness inhibition and autophagy-mediated apoptosis. *Cell Death & Disease* 2022 13:4, 13(4), 400-. <https://doi.org/10.1038/s41419-022-04828-3>
242. Undieh, A. S. (2010). Pharmacology of Signaling Induced by Dopamine D1-Like Receptor Activation. *Pharmacology & Therapeutics*, 128(1), 37. <https://doi.org/10.1016/J.PHARMTHERA.2010.05.003>
243. Urs, N. M., Peterson, S. M., & Caron, M. G. (2016). New Concepts in Dopamine D2 Receptor Biased Signaling and Implications for Schizophrenia Therapy. *Biological Psychiatry*, 81(1), 78. <https://doi.org/10.1016/J.BIOPSYCH.2016.10.011>
244. Vallera, D. A., Oh, F., Kodal, B., Hinderlie, P., Geller, M. A., Miller, J. S., & Felices, M. (2021). A HER2 tri-specific NK cell engager mediates efficient targeting of human ovarian cancer. *Cancers*, 13(16), 3994. <https://doi.org/10.3390/cancers13163994>
245. Vallone, D., Picetti, R., & Borrelli, E. (2000). Structure and function of dopamine receptors. *Neuroscience and Biobehavioral Reviews*, 24(1), 125–132. [https://doi.org/10.1016/S0149-7634\(99\)00063-9](https://doi.org/10.1016/S0149-7634(99)00063-9)
246. Vandenput, I., Debiec-Rychter, M., Capoen, A., Verbist, G., Vergote, I., Moerman, P., & Amant, F. (2011). Kit Gene in Endometrial Carcinoma: An Immunohistochemical and

- Mutational Analysis. *International Journal of Gynecological Cancer*, 21(2), 203–205. <https://doi.org/10.1097/IGC.0b013e3182055c94>
247. Vander Lugt, B., Khan, A. A., Hackney, J. A., Agrawal, S., Lesch, J., Zhou, M., Lee, W. P., Park, S., Xu, M., Devoss, J., Spooner, C. J., Chalouni, C., Delamarre, L., Mellman, I., & Singh, H. (2013). Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation. *Nature Immunology* 2013 15:2, 15(2), 161–167. <https://doi.org/10.1038/ni.2795>
 248. Vidal-Sabanés, M., Bonifaci, N., Navaridas, R., Egea, J., Encinas, M., Rodriguez-Barrueco, R., Silva, J. M., Matias-Guiu, X., Llobet-Navas, D., & Dolcet, X. (2025). Endometrial cancer progression driven by PTEN-deficiency requires miR-424(322)~503. *Cell Death & Disease* 2025 16:1, 16(1), 705-. <https://doi.org/10.1038/s41419-025-08022-z>
 249. Vledder, A., Paijens, S. T., Loiero, D., Maagdenberg, A., Duiker, E. W., Bart, J., Hendriks, A. M., Jalving, M., Werner, N., van Rooij, N., Plat, A., Wisman, G. B. A., Yigit, R., Roelofsen, T., Kruse, A. J., de Lange, N. M., Koelzer, V. H., de Bruyn, M., & Nijman, H. W. (2024). B cells critical for outcome in high grade serous ovarian carcinoma. *International Journal of Cancer*, 155(12), 2265–2276. <https://doi.org/10.1002/ijc.35149>
 250. Volinsky-Fremond, S., Horeweg, N., Andani, S., Barkey Wolf, J., Lafarge, M. W., de Kroon, C. D., Ørtoft, G., Høgdall, E., Dijkstra, J., Jobsen, J. J., Lutgens, L. C. H. W., Powell, M. E., Mileshekin, L. R., Mackay, H., Leary, A., Katsaros, D., Nijman, H. W., de Boer, S. M., Nout, R. A., ... Bosse, T. (2024). Prediction of recurrence risk in endometrial cancer with multimodal deep learning. *Nature Medicine* 2024 30:7, 30(7), 1962–1973. <https://doi.org/10.1038/s41591-024-02993-w>
 251. Wagner, J., Kline, C. L., Ralff, M. D., Lev, A., Lulla, A., Zhou, L., Olson, G. L., Nallaganchu, B. R., Benes, C. H., Allen, J. E., Prabhu, V. V., Stogniew, M., Oster, W., & El-Deiry, W. S. (2017). Preclinical evaluation of the imipridone family, analogs of clinical stage anti-cancer small molecule ONC201, reveals potent anti-cancer effects of ONC212. *Cell Cycle*, 16(19), 1790–1799. <https://doi.org/10.1080/15384101.2017.1325046>
 252. Walsh, C. S., Hacker, K. E., Secord, A. A., DeLair, D. F., McCourt, C., & Urban, R. (2023). Molecular testing for endometrial cancer: An SGO clinical practice statement. *Gynecologic Oncology*, 168, 48–55. <https://doi.org/10.1016/j.ygyno.2022.10.024>
 253. Wang, K. H., Chang, Y. H., Harnod, T., & Ding, D. C. (2022). Endometrial Cancer-Infiltrating Mesenchymal Stem Cells Exhibit Immunosuppressive Effects. *Cell Transplantation*, 31, 09636897221104452. <https://doi.org/10.1177/09636897221104452>
 254. Wang, L., Evans, J. C., Ahmed, L., & Allen, C. (2023). Folate receptor targeted nanoparticles containing niraparib and doxorubicin as a potential candidate for the treatment of high grade serous ovarian cancer. *Scientific Reports* 2023 13:1, 13(1), 1–15. <https://doi.org/10.1038/s41598-023-28424-3>

255. Wang, N., Liu, C., Yao, W., Zhou, H., Yu, S., Chen, H., & Qiao, W. (2021). A traceable, GSH/pH dual-responsive nanoparticles with spatiotemporally controlled multiple drugs release ability to enhance antitumor efficacy. *Colloids and Surfaces B: Biointerfaces*, 205, 111866. <https://doi.org/10.1016/j.colsurfb.2021.111866>
256. Wang, T., Qin, Y., Lai, H., Wei, W., Li, Z., Yang, Y., Huang, M., & Chen, J. (2019). The prognostic value of ADRA1 subfamily genes in gastric carcinoma. *Oncology Letters*, 18(3), 3150–3158. <https://doi.org/10.3892/ol.2019.10660>
257. Wang, T., Yu, D., Wang, J., Zhu, N., Tang, X. Bin, Chen, X., Su, X. M., & Huang, Y. G. (2023). Immune signatures of the POLE mutation in endometrial carcinomas: a systematic study based on TCGA data and clinical cohort validation. *Frontiers in Oncology*, 13, 1250558. <https://doi.org/10.3389/fonc.2023.1250558>
258. Wang, X., Fan, Y., Wang, Q., Shu, X., Lin, J., Guo, J., Li, Z., & Xu, J. (2025). Tumor-infiltrating nerves: unraveling the role of cancer neuroscience in tumorigenesis, disease progression, and emerging therapies. *Discover Oncology*, 16(1), 1209. <https://doi.org/10.1007/s12672-025-02827-2>
259. Wang, X., Zhang, W., Sun, X., Lin, Y., & Chen, W. (2018). Cancer-associated fibroblasts induce epithelial-mesenchymal transition through secreted cytokines in endometrial cancer cells. *Oncology Letters*, 15(4), 5694. <https://doi.org/10.3892/ol.2018.8000>
260. Wang, Y., Liu, N., Guo, X., Han, R., Bai, J., Zhong, J., & Wang, Q. (2025). The immune microenvironment in endometrial carcinoma: mechanisms and therapeutic targeting. *Frontiers in Immunology*, 16, 1586315. <https://doi.org/10.3389/FIMMU.2025.1586315>
261. Wei, Y., Liang, Y., Lin, H., Dai, Y., & Yao, S. (2020). Autonomic nervous system and inflammation interaction in endometriosis-associated pain. *Journal of Neuroinflammation*, 17(1). <https://doi.org/10.1186/s12974-020-01752-1>
262. Weigelt, B., Warne, P. H., Lambros, M. B., Reis-Filho, J. S., & Downward, J. (2013). PI3K pathway dependencies in endometrioid endometrial cancer cell lines. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, 19(13), 3533. <https://doi.org/10.1158/1078-0432.CCR-12-3815>
263. Weissenrieder, J. S., Neighbors, J. D., Mailman, R. B., & Hohl, R. J. (2019). Cancer and the Dopamine D2 Receptor: A Pharmacological Perspective. *The Journal of Pharmacology and Experimental Therapeutics*, 370(1), 111. <https://doi.org/10.1124/JPET.119.256818>
264. Weitz, J., Gulay, K. C. M., de Mendoza, T. H., Tiriach, H., Baumgartner, J., Kelly, K., Veerapong, J., & Lowy, A. M. (2022). Culture and Imaging of *Ex vivo* Organotypic Pseudomyxoma Peritonei Tumor Slices from Resected Human Tumor Specimens. *Journal of Visualized Experiments*, 2022(190). <https://doi.org/10.3791/64620>
265. Weitz, J. R., Tiriach, H., de Mendoza, T. H., Wascher, A., & Lowy, A. M. (2021). Using organotypic tissue slices to investigate the microenvironment of pancreatic cancer: Pharmacotyping and beyond. In *Cancers* (Vol. 13, Number 19). MDPI. <https://doi.org/10.3390/cancers13194991>

266. Wen, S., Lin, X., Luo, W., Pan, Y., Liao, F., Wang, Z., Zhan, B., Feng, J., & Huang, H. (2024). Metabolic difference between patient-derived xenograft model of pancreatic ductal adenocarcinoma and corresponding primary tumor. *BMC Cancer*, 24(1), 485. <https://doi.org/10.1186/s12885-024-12193-x>
267. World Health Organization. Global Cancer Observatory: Cancer Today. Lyon: International Agency for Research on Cancer; 2020. Available from: <https://gco.iarc.fr/today> [Accessed: January 2026]
268. Wijayaratna, D., Ratnayake, K., Ubeyasinghe, S., Kankanamge, D., Tennakoon, M., & Karunaratne, A. (2023). The spatial distribution of GPCR and Gβγ activity across a cell dictates PIP3 dynamics. *Scientific Reports* 2023 13:1, 13(1), 2771-. <https://doi.org/10.1038/s41598-023-29639-0>
269. Wojtyła, C., Bertuccio, P., Giermaziak, W., Santucci, C., Odone, A., Ciebiera, M., Negri, E., Wojtyła, A., & La Vecchia, C. (2023). European trends in ovarian cancer mortality, 1990–2020 and predictions to 2025. *European Journal of Cancer*, 194, 113350. <https://doi.org/10.1016/J.EJCA.2023.113350>
270. Wu, J., Zhang, R., Tang, N., Gong, Z., Zhou, J., Chen, Y., Chen, K., & Cai, W. (2015). Dopamine inhibits the function of Gr-1+CD115+ myeloid-derived suppressor cells through D1-like receptors and enhances anti-tumor immunity. *Journal of Leukocyte Biology*, 97(1), 191–200. <https://doi.org/10.1189/JLB.5A1113-626RR/-/DC1>
271. Wu, L., Deng, Z., Peng, Y., Han, L., Liu, J., Wang, L., Li, B., Zhao, J., Jiao, S., & Wei, H. (2017). Ascites-derived IL-6 and IL-10 synergistically expand CD14+HLA-DR-/low myeloid-derived suppressor cells in ovarian cancer patients. *Oncotarget*, 8(44), 76843. <https://doi.org/10.18632/ONCOTARGET.20164>
272. Wu, Y., Zhu, L., Li, S., Liu, L., Wang, Y., Yang, Y., Mu, Y., Zhu, Q., Jiang, Y., Wu, C., Xi, P., Ma, C., Liang, L., Gao, M., Hu, Y., Ding, Q., & Pan, S. (2025). DA-DRD5 signaling reprograms B cells to promote CD8+ T cell-mediated antitumor immunity. *Cell Reports*, 44(3), 115364. <https://doi.org/10.1016/J.CELREP.2025.115364>
273. Xie, X., Luo, X., Xie, M., Liu, Y., & Wu, T. (2016). Risk of lung cancer in Parkinson's disease. *Oncotarget*, 7(47), 77319. <https://doi.org/10.18632/oncotarget.12964>
274. Xu, Y., Hu, J., Lv, Q., Shi, C., Qiu, M., Xie, L., Liu, W., Yang, B., Shan, W., Cheng, Y., Zhao, B., & Chen, X. (2023). Endometrium-derived mesenchymal stem cells suppress progression of endometrial cancer via the DKK1-Wnt/β-catenin signaling pathway. *Stem Cell Research & Therapy*, 14(1). <https://doi.org/10.1186/s13287-023-03387-4>
275. Yamamoto, T. M., McMellen, A., Watson, Z. L., Aguilera, J., Ferguson, R., Nurmemmedov, E., Thakar, T., Moldovan, G.-L., Kim, H., Cittelly, D. M., Joglar, A. M., Brennecke, E. P., Wilson, H., Behbakht, K., Sikora, M. J., Bitler, B. G., & Benjamin Bitler, C. G. (2019). Activation of Wnt signaling promotes olaparib resistant ovarian cancer. *Molecular Carcinogenesis*, 58, 1770. <https://doi.org/10.1002/mc.23064>
276. Yang, B., Li, X., Zhang, W., Fan, J., Zhou, Y., Li, W., Yin, J., Yang, X., Guo, E., Li, X., Fu, Y., Liu, S., Hu, D., Qin, X., Dou, Y., Xiao, R., Lu, F., Wang, Z., Qin, T., ... Sun, C. (2022a). Spatial

- heterogeneity of infiltrating T cells in high-grade serous ovarian cancer revealed by multi-omics analysis. *Cell Reports Medicine*, 3(12), 100856.
<https://doi.org/10.1016/j.xcrm.2022.100856>
277. Yang, H., Zhang, Z., Li, J., Wang, K., Zhu, W., & Zeng, Y. (2024). The Dual Role of B Cells in the Tumor Microenvironment: Implications for Cancer Immunology and Therapy. *International Journal of Molecular Sciences*, 25(21), 11825.
<https://doi.org/10.3390/ijms252111825>
 278. Yi, H., Lee, G., Park, S., Ha, J., Choi, D., Ko, J., & Ahn, J. (2025). Reconstructing the female reproductive system using 3D bioprinting in tissue engineering. *Materials Today Bio*, 34, 102127. <https://doi.org/10.1016/J.MTBIO.2025.102127>
 279. Yildiz, M., Romano, A., & Xanthouleas, S. (2024). Murine Xenograft Models as Preclinical Tools in Endometrial Cancer Research. *Cancers*, 16(23), 3994.
<https://doi.org/10.3390/CANCERS16233994/S1>
 280. Yong, M., Yu, T., Tian, S., Liu, S., Xu, J., Hu, J., & Hu, L. (2017). DR2 blocker thioridazine: A promising drug for ovarian cancer therapy. *Oncology Letters*, 14(6), 8171.
<https://doi.org/10.3892/OL.2017.7184>
 281. Yu, R., Li, Y., Jiang, R., Dang, C., & Zhai, F. (2025). Impact of sympathetic nervous system on immune evasion in high-grade serous ovarian cancer: a review. *Frontiers in Oncology*, 15, 1644895. <https://doi.org/10.3389/FONC.2025.1644895>
 282. Yu, Y., Chen, T., Zheng, Z., Jia, F., Liao, Y., Ren, Y., Liu, X., & Liu, Y. (2024). The role of the autonomic nervous system in polycystic ovary syndrome. *Frontiers in Endocrinology*, 14, 1295061. <https://doi.org/10.3389/fendo.2023.1295061>
 283. Yuan, Q., Shi, Y., Zhang, Y., Shi, Y., Hussain, Z., Zhao, J., jiang, Y., Qiao, Y., Guo, Y., Lu, J., Dong, Z., Dong, Z., Wang, J., & Liu, K. (2024). Domperidone inhibits cell proliferation via targeting MEK and CDK4 in esophageal squamous cell carcinoma. *Cancer Cell International* 2024 24:1, 24(1), 114-. <https://doi.org/10.1186/s12935-024-03291-8>
 284. Zakarya, R., Howell, V. M., & Colvin, E. K. (2020). Modelling Epithelial Ovarian Cancer in Mice: Classical and Emerging Approaches. *International Journal of Molecular Sciences*, 21(13), 4806. <https://doi.org/10.3390/IJMS21134806>
 285. Zhai, Y., Wu, R., Kuick, R., Sessine, M. S., Schulman, S., Green, M., Fearon, E. R., & Cho, K. R. (2017). High-grade serous carcinomas arise in the mouse oviduct via defects linked to the human disease. *Journal of Pathology*, 243(1), 16–25.
<https://doi.org/10.1002/PATH.4927>
 286. Zhang, K., Xie, X., Zheng, S. L., Deng, Y. R., Liao, D., Yan, H. C., Kang, X., Jiang, H. P., & Guo, S. Q. (2023). Tertiary lymphoid structures in gynecological cancers: prognostic role, methods for evaluating, antitumor immunity, and induction for therapy. *Frontiers in Oncology*, 13. <https://doi.org/10.3389/fonc.2023.1276907>
 287. Zhang, L., Cascio, S., Mellors, J. W., Buckanovich, R. J., & Osmanbeyoglu, H. U. (2024). Single-cell analysis reveals the stromal dynamics and tumor-specific characteristics in the microenvironment of ovarian cancer. *Communications Biology*, 7(1):20.
<https://doi.org/10.1038/s42003-023-05733-x>

288. Zhang, M., Chen, Z., Wang, Y., Zhao, H., & Du, Y. (2022). The Role of Cancer-Associated Fibroblasts in Ovarian Cancer. *Cancers*, 14(11), 2637. <https://doi.org/10.3390/CANCERS14112637>
289. Zhang, W., Ding, Y., He, H., Chen, K., Zeng, Q., Cao, X., Xiang, Y., & Zeng, H. (2025). Prospects and challenges of ovarian cancer organoids in chemotherapy research (Review). *Oncology Letters*, 29(4). <https://doi.org/10.3892/OL.2025.14944/DOWNLOAD>
290. Zhang, X., Liu, Q., Liao, Q., & Zhao, Y. (2017). Potential Roles of Peripheral Dopamine in Tumor Immunity. *Journal of Cancer*, 8(15), 2966. <https://doi.org/10.7150/JCA.20850>
291. Zhang, X., Tsuboi, D., Funahashi, Y., Yamahashi, Y., Kaibuchi, K., & Nagai, T. (2022). Phosphorylation Signals Downstream of Dopamine Receptors in Emotional Behaviors: Association with Preference and Avoidance. *International Journal of Molecular Sciences*, 23(19), 11643. <https://doi.org/10.3390/ijms231911643>
292. Zhang, Y., & Tian, J. (2024). Strategies, Challenges, and Prospects of Nanoparticles in Gynecological Malignancies. *ACS Omega*, 9(36), 37459. <https://doi.org/10.1021/acsomega.4c04573>
293. Zhao, B., Chen, S., Hong, Y., Jia, L., Zhou, Y., He, X., Wang, Y., Tian, Z., Yang, Z., & Gao, D. (2022). Research Progress of Conjugated Nanomedicine for Cancer Treatment. *Pharmaceutics*, 14(7), 1522. <https://doi.org/10.3390/PHARMACEUTICS14071522>
294. Zhao, L., Li, X., Jing, Y., Tang, L., Lin, F., Zhang, Y., Tang, Y., Chen, C., Yang, J., Liu, X., & Chen, J. (2025). Biomarkers and immunotherapy in endometrial cancer: mechanisms and clinical applications. *Frontiers in Immunology*, 16, 1684549. <https://doi.org/10.3389/fimmu.2025.1684549>
295. Zhao, L., Zhao, X., Shen, D., Li, X., Wang, J., Wang, H., & Wei, L. (2012). Tumor-Infiltrating Dendritic Cells May Be Used as Clinicopathologic Prognostic Factors in Endometrial Carcinoma. *International Journal of Gynecological Cancer*, 22(5), 836–841. <https://doi.org/10.1097/IGC.0b013e31825401c6>
296. Zhao, W., Huang, Y., Liu, Z., Cao, B. B., Peng, Y. P., & Qiu, Y. H. (2013). Dopamine Receptors Modulate Cytotoxicity of Natural Killer Cells via cAMP-PKA-CREB Signaling Pathway. *PLoS ONE*, 8(6), e65860. <https://doi.org/10.1371/JOURNAL.PONE.0065860>
297. Zhao, Y., He, M., Cui, L., Gao, M., Zhang, M., Yue, F., Shi, T., Yang, X., Pan, Y., Zheng, X., Jia, Y., Shao, D., Li, J., He, K., & Chen, L. (2020). Chemotherapy exacerbates ovarian cancer cell migration and cancer stem cell-like characteristics through GLI1. *British Journal of Cancer* 2020 122:11, 122(11), 1638–1648. <https://doi.org/10.1038/s41416-020-0825-7>
298. Zheng, X., Song, X., Zhu, G., Pan, D., Li, H., Hu, J., Xiao, K., Gong, Q., Gu, Z., Luo, K., & Li, W. (2024). Nanomedicine Combats Drug Resistance in Lung Cancer. *Advanced Materials*, 36(3). <https://doi.org/10.1002/adma.202308977>
299. Zhou, Y., Irwin, M. L., Ferrucci, L. M., McCorkle, R., Ercolano, E. A., Li, F., Stein, K., & Cartmel, B. (2016). Health-Related Quality of Life in Ovarian Cancer Survivors: Results

- from the American Cancer Society's Study of Cancer Survivors-I. *Gynecologic Oncology*, 141(3), 543. <https://doi.org/10.1016/J.YGYNO.2016.04.006>
300. Zhu, B., Gu, H., Mao, Z., Beeraka, N. M., Zhao, X., Anand, M. P., Zheng, Y., Zhao, R., Li, S., Manogaran, P., Fan, R., Nikolenko, V. N., Wen, H., Basappa, B., & Liu, J. (2024). Global burden of gynaecological cancers in 2022 and projections to 2050. *Journal of Global Health*, 14. <https://doi.org/10.7189/JOGH.14.04155>
301. Zhu, H., Shao, J., Shao, L., Cai, L., Gu, C., Ge, Q., & Liu, J. (2025). Immunotherapy strategies targeting tumor-associated macrophages and their mechanisms of action in tumor progression. *Frontiers in Immunology*, 16, 1680455. <https://doi.org/10.3389>

Scientific Contributions

Scientific Publications (See Annex)

- Edwards, M. et al. (2026). Shaping the future one slice at a time: How 3D organotypic tumour slice models are driving drug discovery in ovarian cancer. *Translational Oncology*, 63:102633.
- Revilla et al. (2023). Lenvatinib-loaded poly(lactic-co-glycolic acid) nanoparticles with epidermal growth factor receptor antibody conjugation... *Biomolecules*, 13(11):1647.
- Español et al. (2023). Dopamine receptors D1 and D2 show prognostic significance and potential therapeutic applications for endometrial cancer patients. *Gynecologic Oncology*, 176(3):25-35.

Conference Contributions and Posters

- ICONN Sydney (2026): Poster Presentation. *Cutting-edge slices: An ex vivo 3D tumour platform for dopaminergic nanomedicine testing*.
- ESMO Immuno-Oncology Congress (2025): Poster Presentation (383P). *Blocking dopamine D2 receptors induces anti-cancer and immune stimulatory effects...*
- ESCI Barcelona (2024): Poster Presentation. *Validating organotypic tumour slice culture as a 3D drug evaluation platform...*
- ICONAN Barcelona (2024): Co-author Poster. *RNase enzyme functionalized vaults for the treatment of pancreatic cancer*.
- PMWC Silicon Valley (2024): Professional Attendance at the Precision Medicine World Conference.

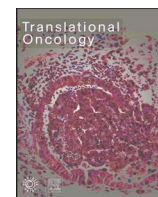
Training and Academic Activities

- Biocat Design Health (2025-2026): 8-month healthcare innovation and entrepreneurship program using Stanford's Biodesign methodology to solve unmet clinical needs.
- SIRIS Academic Doctoral Training: Completed three years of the INPhINIT Fellowship training programme (2022-2025).

Awards and Grants

- Best Poster Prize (2026): Awarded the Royal Society of Chemistry Pharmaceutics prize at ICONN26 Sydney.
- 'Shoot your PhD' Contest: Winner of the La Caixa Foundation contest for the video *"Can we repurpose dopamine-related drugs to fight ovarian cancer?"*.
- Immuno-model COST Action (2025): Awarded the Young Researcher and Innovator Conference Grant.

Annexes



Shaping the future one slice at a time: How 3D organotypic tumour slice models are driving drug discovery in ovarian cancer

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ABSTRACT

Despite therapeutic advancements, ovarian cancer remains one of the most lethal gynaecological malignancies, highlighting the urgent need for innovative drug discovery approaches. Traditional 2D cell line models fail to accurately replicate the complexity of the tumour and its microenvironment (TME), leading to suboptimal drug evaluations. Organotypic tumour slice culture (OTSC) has emerged as a promising 3D *ex vivo* platform that preserves native tissue architecture, cellular interactions, and molecular heterogeneity of the tumour and its TME, providing a more physiologically relevant system for drug testing. This review examines the evolution of OTSC in ovarian cancer and its applications across chemotherapy, targeted therapy, immunotherapy, and virotherapy. We highlight the role of OTSC in identifying predictive biomarkers of drug response, drug resistance mechanisms – including those in the stroma – and elucidating novel therapeutic strategies. While OTSC has demonstrated its ability to rapidly provide insights into patient-specific responses, further integration of advanced multi-omics analyses could enhance its potential as a precision medicine platform. With its 3D heterogenic complexity, OTSC could also facilitate the development and evaluation of TME-directed therapies, novel multitarget drugs, and tumour-targeting drug delivery systems, ultimately shaping the future of ovarian cancer therapy and improving clinical outcomes.

Introduction

Ovarian cancer is the 8th most prevalent malignancy in women and considered one of the most lethal gynaecological cancers [1]. Despite advancements in targeted treatments, such as PARP inhibitors, Bevacizumab, and Mirvetuximab soravtansine-gynx, and the integration of complementary predictive molecular biomarkers, still over 70 % of patients face disease recurrence and a 5-year survival rate of 30 % [1–3].

While there is a clear, unmet need for new clinically approved therapeutics, the research community are battling high drug development costs, timeframes, and attrition rates, with approximately 95 % of new anti-cancer agents failing at clinical phase I trials [4]. One important facet to the high clinical failings is the use of unsuitable drug discovery models for pharmaceutical screenings, for instance using simplistic 2D monolayers devoid of the native tumour heterogeneity and complexity for preclinical drug testing and selection that ultimately failure to accurately predict clinical translation [5]. Recently, 3D

organotypic tumour slice culture (OTSC) has received increased interest as a drug discovery model [4]. This technique thinly slices and cultures resected tumour material from patient biopsy or xenografted animal models to rapidly create a precision drug testing platform [6]. A major advantage of the 3D OTSC model is its ability to preserve the native tumour microenvironment (TME), including cancer cell-cell and cell-matrix interactions. This is particularly important for studying high-grade serous ovarian cancer (HGSOC), which features four molecular subtypes varying by stroma, immune activation, and cell differentiation [7]. In this context, maintaining the original stroma, immune, and vascular networks are crucial for understanding disease mechanisms, drug resistance, and therapeutic efficacy [8]. Moreover, through utilisation of patient-derived biopsy, OTSC can capture patient-specific drug responses, assess therapeutic efficacy, and identify resistance mechanisms, making it a promising platform for both pre-clinical drug development and personalised medicine approaches in clinical settings [9].

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Article

Lenvatinib-Loaded Poly(lactic-co-glycolic acid) Nanoparticles with Epidermal Growth Factor Receptor Antibody Conjugation as a Preclinical Approach to Therapeutically Improve Thyroid Cancer with Aggressive Behavior

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Abstract: Background: Lenvatinib, a tyrosine kinase inhibitor (TKI) approved for the treatment of progressive and radioactive iodine (RAI)-refractory differentiated thyroid cancer (DTC), is associated with significant adverse effects that can be partially mitigated through the development of novel drug formulations. The utilization of nanoparticles presents a viable option, as it allows for targeted drug delivery, reducing certain side effects and enhancing the overall quality of life for patients. This study aimed to produce and assess, both in vitro and in vivo, the cytotoxicity, biodistribution, and therapeutic efficacy of lenvatinib-loaded PLGA nanoparticles (NPs), both with and without decoration using antibody conjugation (cetuximab), as a novel therapeutic approach for managing aggressive thyroid tumors. Methods: Poly(lactic-co-glycolic acid) nanoparticles (NPs), decorated with or without anti-EGFR, were employed as a lenvatinib delivery system. These NPs were characterized for size distribution, surface morphology, surface charge, and drug encapsulation efficiency. Cytotoxicity was evaluated through MTT assays using two cellular models, one representing normal thyroid cells (Nthy-ori 3-1) and the other representing anaplastic thyroid cells (CAL-62). Additionally, an in vivo xenograft mouse model was established to investigate biodistribution and therapeutic efficacy following intragastric administration. Results: The NPs demonstrated success in terms of particle size, polydispersity index (PDI), zeta potential, morphology, encapsulation efficiency, and cetuximab distribution across the surface. In vitro analysis revealed cytotoxicity in both cellular models with both formulations, but only the decorated NPs achieved an ID50 value in CAL-62



Dopamine receptors D1 and D2 show prognostic significance and potential therapeutic applications for endometrial cancer patients

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HIGHLIGHTS

- ADR β 2 expression was neither associated with clinico-pathological parameters nor prognostic value in the analyzed patients.
- DRD1 expression showed an inverse association with tumor size and stage in our endometrial cancer patient cohort.
- DRD2 expression showed significant positive association with non-endometrioid endometrial cancer, high grade and tumor size.
- High expression of DRD2 is an independent significant prognostic marker for predicting OS and DFS in the patient's cohort.
- DRD1 agonism and DRD2 antagonism combination reduced cellular viability and could have a potential therapeutic benefit.

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ABSTRACT

Objective. Catecholaminergic signaling has been a target for therapy in different type of cancers. In this work, we characterized the ADR β 2, DRD1 and DRD2 expression in healthy tissue and endometrial tumors to evaluate their prognostic significance in endometrial cancer (EC), unraveling their possible application as an antitumor therapy.

Methods. 109 EC patients were included. The expression of the ADR β 2, DRD1 and DRD2 proteins was evaluated by immunohistochemistry and univariate and multivariate analysis to assess their association with clinic-pathological and outcome variables. Finally, HEC1A and AN3CA EC cell lines were exposed to different concentrations of selective dopaminergic agents alone or in combination to study their effects on cellular viability.

Results. ADR β 2 protein expression was not associated with clinico-pathological parameters or prognosis. DRD1 protein expression was reduced in tumors samples but showed a significant inverse association with tumor size and stage. DRD2 protein expression was significantly associated with non-endometrioid EC, high grade tumors, tumor size, worse disease-free survival (HR = 3.47 (95%CI:1.35–8.88)) and overall survival (HR = 2.98 (95%CI:1.40–6.34)). The DRD1 agonist fenoldopam showed a reduction of cellular viability in HEC1A and AN3CA cells. The exposure to domperidone, a DRD2 antagonist, significantly reduced cell viability compared to the control. Finally, DRD1 agonism and DRD2 antagonism combination induced a significant

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INFORME D'AVALUACIÓ D'UN PROJECTE PER LA COMISSIÓ D'EXPERIMENTACIÓ ANIMAL COM A ÒRGAN HABILITAT PER LA GENERALITAT DE CATALUNYA (Reial Decret 53/2013, d'1 de febrer)
Ref. núm. de projecte: 10234
Títol del projecte: Evaluación de nanoconjugados para terapia dirigida en modelos murinos de cáncer de páncreas, endometrio y glioblastoma
Projecte tipus: ☐ I ☐ II ☒ III
Persona responsable del projecte: Maria Virtudes Céspedes Navarro
Data d'admissió de la documentació: 16.07.2018 / 18.10.2018 / 04.02.2019 / 25.02.2019
Data d'avaluació per la CEA com a OH: 12.09.2018 / 07.11.2018 / 30.01.2019 / 20.03.2019

Revisada la documentació presentada, s'informen, d'acord amb la normativa vigent*, els punts següents:

1. Avaluada la informació relativa a la procedència dels animals (art.19 del RD 53/2013 d'1 de febrer i només per a les espècies* incloses a l'annex I del RD 53/2013 d'1 de febrer o a l'annex de la Llei 5/1995, de 21 de juny), s'informa,

(*) la utilització de gossos i gats rodamóns, i els provinents de centres de recollida d'animals abandonats està prohibida a Catalunya (art. 4.1.a de la Llei 5/1995)

☐ No procedeix, atès que l'espècie animal no està inclosa a l'annex I del RD 53/2013, d'1 de febrer.

☒ Favorablement

☐ Desfavorablement, atès que

Observacions/Justificació:

2. Avaluada la informació presentada relativa a la utilització de primats no humans en el projecte, d'acord amb l'art. 21 del RD 53/2013 d'1 de febrer, s'informa,

☒ No procedeix, atès que no s'utilitzen primats no humans.

☐ Favorablement.

☐ Desfavorablement, atès que

Observacions/Justificació:

3. Avaluada la informació relativa a l'ús d'anestèsia durant el projecte (art. 26 del RD 53/2013 d'1 de febrer), s'informa,

☐ No procedeix, atès que el projecte no ho requereix.

☒ Favorablement el protocol d'anestèsia a aplicar.

☐ Favorablement la justificació presentada pel responsable del projecte en relació amb la no utilització de l'anestèsia per ser incompatible amb el projecte o per estar contraindicada.

☐ Desfavorablement, atès que

Observacions/Justificació:

1

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GENERALITAT DE CATALUNYA



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12/04/2024 00:00:00
Pàgina 1 de 5

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4. Avaluada la informació relativa a l'ús d'analgèsia o altres mètodes destinats a eliminar al màxim el dolor, el sofriment durant el projecte (art. 26 del RD 53/2013 d'1 de febrer), s'informa,

☐ No procedeix, atès que el projecte no ho requereix.

☒ Favorablement el protocol d'analgèsia o altres mètodes a aplicar destinats a eliminar al màxim el dolor.

☐ Favorablement la justificació presentada pel responsable del projecte en relació amb la no utilització de l'analgèsia per ser incompatible amb el projecte o per estar contraindicada.

☐ Desfavorablement, atès que

Observacions/Justificació:

5. Avaluada la informació relativa a les condicions d'allotjament, zootècniques i de cura dels animals en el projecte (art. 6 del RD53/2013 d'1 de febrer), s'informa,

☐ No procedeix, atès que el projecte no ho requereix.

☒ Favorablement.

☐ Desfavorablement, atès que

Observacions/Justificació:

6. Mètode d'eutanàsia:

6.1. Avaluada la informació relativa al mètode d'eutanàsia (art. 7 i annex III del RD 53/2013 d'1 de febrer) en finalitzar el projecte, s'informa,

☐ No procedeix, atès que els animals no són eutanasiats

☒ Favorablement

☐ Desfavorablement, atès que

Observacions/Justificació:

6.2. I en relació amb el mètode d'eutanàsia en aplicació a criteris de punt final, s'informa,

☐ No procedeix, atès que el projecte no ho requereix.

☒ Favorablement.

☐ Desfavorablement, atès que

Observacions/Justificació:

7. Avaluades les condicions generals previstes en l'execució del projecte (art. 25.3 del RD 53/2013 d'1 de febrer) s'informa,

☒ Favorablement, atès que el projecte es realitzaria tenint en compte evitar als animals qualsevol dolor, sofriment, angoixa o dany durador innecessari.

☐ Desfavorablement, atès que

Observacions/Justificació:

2

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12/04/2019 11:30:23
Data caducitat còpia:
12/04/2024 00:00:00
Pàgina 2 de 5

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Comissió d'Experimentació Animal
Òrgan Habilitat

Ref. núm. informe: CEA-OH/10234/4

8. Avaluada la informació presentada en relació amb l'acreditació/capacitació del personal que participa en el projecte (art. 25.5 del RD 53/2013 d'1 de febrer), s'informa,

☒ Favorablement.

☐ Desfavorablement, atès que
Observacions/Justificació:

9. Avaluada la informació relacionada amb el transport dels animals (art. 9.1 del RD 53/2013 d'1 de febrer), s'informa,

☒ No procedeix, atès que els animals no són transportats durant el projecte.

☐ Favorablement.

☐ Desfavorablement, atès que
Observacions/Justificació:

10. Avaluada la informació relativa a la utilització d'animals d'espècies amenaçades (art. 20 del RD 53/2013 d'1 de febrer), s'informa,

☒ No procedeix

☐ Favorablement.

☐ Desfavorablement, atès que
Observacions/Justificació:

11. Avaluada la informació relativa a la utilització d'animals capturats en la natura (art. 22 del RD 53/2013 d'1 de febrer), s'informa,

☒ No procedeix.

☐ Favorablement.

☐ Desfavorablement, atès que
Observacions/Justificació:

12. Avaluada la informació relativa a la reutilització d'animals (utilització d'animals que han estat sotmesos prèviament a un altre projecte) (art. 29 del RD 53/2013 d'1 de febrer), s'informa,

☒ No procedeix.

☐ Favorablement.

☐ Desfavorablement, atès que
Observacions/Justificació:

13. Avaluada la finalitat, els beneficis científics i, si s'escau, el valor docent del projecte (art. 34.2a del RD 53/2013 d'1 de febrer) s'informa,

☒ Favorablement.

☐ Desfavorablement, atès que
Observacions/Justificació:

3

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12/04/2019 11:30:23
Data caducitat còpia:
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Pàgina 3 de 5

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Òrgan Habilitat

Ref. núm. informe: CEA-OH/10234/4

14. Avaluada la conformitat amb les 3Rs (art.34.2b del RD 53/2013 d'1 de febrer), s'informa,

☒ Favorablement.

☐ Desfavorablement.

Observacions/Justificació:

15. Avaluat el grau de severitat indicat a la memòria del projecte (art.34.2c del RD 53/2013 d'1 de febrer) s'informa,

☒ Favorablement.

☐ Desfavorablement, atès

Observacions/Justificació:

Amb la classificació de:

☐ Sense recuperació

☐ Lleu

☐ Moderat

☒ Sever

16. Balanç ètic: analitzats els danys i beneficis del projecte (art.34.2d del RD 53/2013 d'1 de febrer), s'informa,

☒ Favorablement.

☐ Desfavorablement.

Observacions/Justificació:

17. El projecte requereix un resum no tècnic (art. 33.1 del RD 53/2013 d'1 de febrer).

☒ Sí

☐ No

Atès que és de Tipus III

18. Avaluació retrospectiva del projecte (art. 35.1 del RD 53/2013 d'1 de febrer).

Atès que el projecte,

☐ Utilitza primats.

☐ S'utilitzen primats per a finalitats diferents a les previstes a l'art. 21.2.a del RD 53/2013 d'1 de febrer.

☒ Ha estat classificat com a sever.

☐ El projecte implica un dolor greu i perllongat que no pot ser alleujat.

☐ Altres: Article 33.6 f) del RD 53/2013

Justificació:

S'informa que el projecte,

☐ No requereix avaluació retrospectiva.

☒ Sí, cal realitzar l'avaluació retrospectiva la qual s'ha de presentar al finalitzar el projecte o com a màxim als 5 anys de l'autorització d'aquest.

19. Altres comentaris:

4

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Pàgina 4 de 5

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Comissió d'Experimentació Animal
Òrgan Habilitat

Ref. núm. informe: CEA-OH/10234/4

RESULTAT FINAL DE L'INFORME.

Per tant, tenint en compte l'informe de cadascun dels punts anteriors,

☒ S'informa FAVORABLEMENT

☐ S'informa DESFAVORABLEMENT

Justificació:

Sílvia Leonart
Secretària

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5



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12/04/2024 00:00:00
Pàgina 5 de 5