

A Translational Approach to Clinical Data and Molecular Profiling in Ovarian Cancer



PhD Thesis 2026

by

Dr Ciara McNevin

SUPERVISOR:

Prof Stephen Finn

Department of Histopathology

Trinity College Dublin/

St James's Hospital

CO SUPERVISOR:

Prof Karen Cadoo

Department of Oncology

Trinity College Dublin/

St James's Hospital

Declaration

I have read and I understand the plagiarism provisions in the General Regulations of the University Calendar for the current year, found at <https://libguides.tcd.ie/academic-integrity/>. I

have completed the Online Tutorial in avoiding plagiarism 'Ready, Steady, Write', located at https://www.tcd.ie/library/support/plagiarism/story_html5.html



Dr Ciara McNevin

Summary

Ovarian cancer (OC) remains one of the most lethal gynaecological malignancies, characterised by late-stage presentation, intrinsic genomic instability, and frequent development of treatment resistance. Although the identification of cancer-predisposing genetic alterations (CPGAs) such as *BRCA1* and *BRCA2* has transformed therapeutic strategies, significant challenges persist in linking molecular mechanisms of resistance to clinical outcomes.

In this thesis, we investigated the genomic and transcriptomic determinants of hereditary and treatment-resistant OC and developed the foundational infrastructure to support genomic research in Ireland. Our integrated approach combined clinical cohort analysis, in vitro modelling, and registry-based data collection to bridge translational and real-world insights.

We first characterised the prevalence and clinical implications of *BRCA1* and *BRCA2* pathogenic variants in Irish OC cohorts using paired germline and somatic testing data. We demonstrated the feasibility and clinical value of unified testing across Irish cancer centres, providing the first national dataset describing *BRCA1/2*-associated OC. These findings informed ongoing national policy development in genomic medicine.

We then developed chemotherapy- and PARP inhibitor (PARPi)-resistant OC cell line models across *BRCA1*-mutant, *BRCA2*-mutant, and *BRCA*-wildtype backgrounds through stepwise drug exposure reflecting clinically relevant plasma concentrations. Using circRNA and miRNA profiling, we identified over 4,000 differentially expressed circRNAs and prioritised five circRNA candidates (*hsa_circ_400223*, *hsa_circ_003300*, *hsa_circ_008026*, *hsa_circ_100059*, and *hsa_circ_025636*) for validation. Functional enrichment analysis revealed dysregulation of DNA repair, chromatin organisation, and intracellular signalling pathways, implicating these non-coding RNAs in mechanisms of acquired chemoresistance. We further identified miRNA regulators differentially expressed by *BRCA* status, including miR-27b-3p, miR-205-5p, and miR-590-3p, suggesting potential utility as molecular signatures of tumour phenotype and therapeutic response. Validation across cell line models and clinical samples confirmed the expression trends of selected circRNA candidates, providing proof-of-concept for their biomarker potential.

In parallel, we devised and implemented the CAmPaiGn pilot registry—the first Irish registry for individuals with CPGAs—establishing a framework for structured data collection across demographic, clinical, and genomic domains. Through national survey work and direct patient and public involvement (PPI), we demonstrated strong public willingness to contribute data for research and clinical integration, underscoring feasibility for a future national hereditary cancer registry.

Collectively, this work provides new molecular insights into mechanisms of resistance in BRCA-mutant OC, identifies circRNA and miRNA candidates with biomarker potential, and establishes the infrastructure required to translate these findings into clinical practice. By combining in silico, in vitro, and clinical methodologies, we demonstrated how molecular discovery can inform predictive, personalised cancer care and shape the future of precision oncology in Ireland.

Acknowledgements

This PhD was funded through the Irish Clinical Academic Training (ICAT) Programme, supported by the Health Research Board (HRB) and the Wellcome Trust. I am deeply grateful for this opportunity and for the training, mentorship, and community it provided. A special thank you to Karen Misstear in ICAT for her endless support, organisation, and unfailing good humour. Thanks also to Prof McDermott for his kind mentoring, and importantly for directing me to Prof Finn's lab — without this, things would have evolved very differently, and certainly not as well or half as enjoyably.

I owe enormous (and probably unquantifiable) gratitude to Dr Anne-Marie Baird and Dr Steven Gray for their exceptional expertise, generosity of time, practicality, and level-headedness. I am equally thankful to Professor Cadoo for her always excellent input, depth of knowledge and kind support, and to Professor Finn for his steady patience, wisdom, humour and unflappable calm. My sincere thanks to all of you - through countless meetings, impromptu guidance, and shared expertise, you have helped me in the most tangible of ways.

To everyone in TTMI, with special thanks to Dr Sharon O'Toole, Dr Martin Barr, Faye Lewis, and Dr Mark Ward — you are wonderful people doing truly important work. To all in Cancer Molecular Diagnostics (CMD) across St James's and Beaumont Hospitals, thank you for your skill, efficiency, and collaboration in bringing projects across the line. You provide an exceptional service whose impact is often unseen but deeply significant.

I have genuinely enjoyed this PhD, largely because of the people I have had the privilege to share the SPD lab with — Megan O'Shaughnessy, Josh Flynn, Pierre Murchan, Vicky O'Donachie, and the many Masters students who brought energy, enthusiasm, and endless entertainment to our days. Thank you to Oana Deac, my fellow oncology SpR, ICAT colleague, mother of two, and conference date always.

To the clinical oncology consultants who have supported and mentored me during my time in St James's Hospital - a huge thank you. A special mention goes to Dr Grant, my clinical trainer, for unwavering support, kindness and guidance throughout this journey.

To my parents, sisters, and wonderful in-laws - thank you for your loyalty, commitment, and the constant safety net you provide. Your childcare back-up, steady presence, and support have been irreplaceable. I quite literally could not have done this without you.

To my incredible friends - the many fractions of friendship that quietly (and sometimes loudly) cheer me on, thank you for always being there. You have celebrated my wins, cursed my foes, and hugged me tightly when needed. We have chatted over tea, drunk wine, clinked pints and

sampled the finest food in Dublin. You have so often been the glimmer in a bad week, making me laugh until my sides hurt, and I appreciate you all most dearly.

Lastly, to my most beautiful, most precious, most handsome two boys, Matt and Jack. You have borne the brunt of this sacrifice and are undoubtedly my proudest achievement of the past three years. I hope one day my work makes you proud.

And finally, to Fran - I know you are as excited as I am to hand in this thesis, and you will be the first person I celebrate with! What an adventure these last few years have been; I can't believe how much we've accomplished together. Thank you for your endless support, and thank you for loving me in the sweet and patient way you do.

Figures

Figure 1-1 Female Reproductive System	2
Figure 1-2 AJCC Prognostic Groups	4
Figure 1-3 OC incidence across Europe.....	8
Figure 1-4 Percent Point Improvement in 5 year net survival: 1994 - 1998 vs. 2014 – 2018.....	9
Figure 1-5 Repair Pathways for DSBs	11
Figure 1-6 Genetic breakdown in HGSOC	13
Figure 1-7 The p53/miR-34 pathway regulates apoptosis and is altered in cancer	16
Figure 1-8 Functions of miRNAs in EOC metastasis	18
Figure 1-9 Schematic illustration of platinum resistance	30
Figure 1-10 Schematic illustration of paclitaxel resistance.	32
Figure 1-11 Prevalence of BRCA reversion mutations in SOLO3 Trial	34
Figure 1-12 Schematic illustration of PARPI resistance	35
Figure 2-1 Scheme for linear poly(A) tailed RT-PCR.....	42
Figure 2-2 Cell Line Preparation PCR conditions for mycoplasma testing.....	46
Figure 2-3 Cell Line Preparation and RNA Extraction for circRNA Analysis	52
Figure 2-4 Primer design for circRNA validation.	53
Figure 3-1 BRCA Testing Outcomes: Venn and CONSORT Diagrams	61
Figure 3-2 Density plots of age distribution in Germline/Somatic BRCA1/2 vs. wildtype.....	64
Figure 3-3 Geographical distribution of most common <i>BRCA</i> PV's in Ireland	66
Figure 3-4 BRCA1/2 Mutation Mapping with Lollipop Plots.....	70
Figure 3-5 Regression Analysis of Age at Diagnosis by Histologic Subtype in Young-Onset OC.....	74
Figure 3-6 Kaplan-Meier Curve for OS	75
Figure 4-1 eHealth Scores in the EU : access to eHealth records	90
Figure 4-2 Barriers to Genetic Data Integration in Ireland	93
Figure 4-3 Stakeholder Engagement in Registry Development	95
Figure 4-4 Willingness to Share Health and Genetic Data (n = 50).....	109
Figure 4-5 Registry Priorities and Research Linkage (n = 50).....	110
Figure 5-1 Venn diagram illustrating the selection conditions of miRNAs	118
Figure 5-2 Consort Diagram showing selection process from miRNA in KMPlotter.....	121
Figure 5-3 Survival associated with HMLA2 in OC	130
Figure 5-4 Survival associated with TNFAIP8 in OC	131
Figure 5-5 Survival associated with BICC1 in OC.....	132
Figure 5-6 Survival associated with PRRX1 in OC.....	133
Figure 5-7 miRNA let-7a-3p Forward Primer 1	135
Figure 5-8 hsa-miR-590-3p Forward Primer 1.....	135
Figure 5-9 hsa-miR-590-3p Forward Primer 2.....	136
Figure 5-10 hsa-miR-27b-3p Forward Primer 1	136
Figure 5-11 hsa-miR-27b-3p Forward Primer 2	136
Figure 5-12 hsa-let-7f-5p Forward Primer 1	137
Figure 5-13 hsa-miR-98-5p Forward Primer 1.....	137
Figure 5-14 hsa-miR-205-5p Forward Primer 1.....	137
Figure 5-15 hsa-miR-205-5p Forward Primer 2.....	138
Figure 5-16 hsa-miR-3176 FP1	138
Figure 5-17 hsa-miR-3176 FP2	138
Figure 5-18 hsa-miR-3613-5p FP1	139
Figure 5-19 hsa-miR-652-3p FP1	139

Figure 5-20 Histone H3 (Normalization control)	139
Figure 5-21 18s rRNA housekeeping gene	140
Figure 5-22 miRNA expression profiles between cell line	141
Figure 5-23 miRNA expression profiles between cell line	142
Figure 5-24 BRCA1-related miRNA network. Taken from Chang et al. (374).	146
Figure 6-1 Cov362 photographed at 4x magnification	155
Figure 6-2 Cov362 photographed at 40x magnification	155
Figure 6-3 OVSAHO photographed at 10x magnification	156
Figure 6-4 OVSAHO photographed at 40x magnification	156
Figure 6-5 Kuramochi photographed at 4x magnification.....	157
Figure 6-6 Kuramochi photographed at 40x magnification.....	157
Figure 6-7 Mycoplasma Gel Showing COV362 Positivity.....	158
Figure 6-8 Pre and Post treatment mycoplasma PCR results for COV362 cell line	158
Figure 6-9 Human Cell Line Authentication Summary	159
Figure 6-10 RNA Integrity Confirmed by 18S and 28S Bands	161
Figure 6-11 Hierarchical Clustering Heatmaps of all target values	162
Figure 6-12 Hierarchical Clustering Heatmaps COV362 vs. OVSAHO	163
Figure 6-13 Hierarchical Clustering Heatmaps KURA vs. OVSAHO	164
Figure 6-14 Hierarchical Clustering Heatmaps COV362 vs. KURA.....	164
Figure 6-15 Scatter plots of circRNA expression in BRCA-related cell line comparisons	165
Figure 6-16 Volcano plots of circRNA expression in BRCA-related cell line comparisons	166
Figure 6-17 Top 10 enriched BPs in upregulated circRNAs (COV362 vs OVSAHO)	168
Figure 6-18 Top 10 enriched CCs in upregulated circRNAs (COV362 vs OVSAHO)	169
Figure 6-19 Top 10 enriched MF upregulated in circRNAs (COV362 vs OVSAHO)	170
Figure 6-20 Top 10 enriched BPs in upregulated circRNAs (KURAMOCHI vs OVSAHO).....	171
Figure 6-21 Top 10 enriched CCs in upregulated circRNAs (KURAMOCHI vs OVSAHO).....	172
Figure 6-22 Top 10 enriched BPs in upregulated circRNAs (KURAMOCHI vs OVSAHO).....	173
Figure 6-23 Top 10 enriched BPs in upregulated circRNAs (COV362 vs KURAMOCHI).....	174
Figure 6-24 Top 10 enriched CCs in upregulated circRNAs (COV362 vs KURAMOCHI	175
Figure 6-25 Top 10 enriched MFs in upregulated circRNAs (COV362 vs KURAMOCHI).....	176
Figure 6-26 KEGG pathway of upregulated circRNAs (COV362 vs OVSAHO)	178
Figure 6-27 KEGG pathway of upregulated circRNAs (KURAMOCHI vs OVSAHO)	179
Figure 6-28 KEGG pathway of upregulated circRNAs (COV362 vs KURAMOCHI)	180
Figure 6-29 Chromosomal distribution of circRNAs in COV362 vs OVSAHO	181
Figure 6-30 Chromosomal distribution of circRNAs in KURAMOCHI vs OVSAHO	182
Figure 6-31 Chromosomal distribution circRNAs in COV362 vs KURAMOCHI	183
Figure 6-32 Overlap of Upregulated circRNAs Across Pairwise Comparisons.....	184
Figure 6-33 (A) hsa_circ_400223 forward primer, (B) hsa_circ_400223 reverse primer	190
Figure 6-34 hsa_circ_008026.....	190
Figure 6-35 hsa_circ_0025636.....	191
Figure 6-36 hsa_circ_0100059.....	191
Figure 6-37 hsa_circ_003300.....	191
Figure 6-38 Endpoint PCR demonstrating nonspecific amplification in <i>BRCA</i> WT.....	193
Figure 6-39 Validation of circRNAs in cell lines using qPCR.....	195
Figure 6-40 Fold-change expression of circRNAs across OC cell lines.....	197
Figure 6-41 Validation of circRNA in Clinical Samples	199
Figure 6-42 circInteractome summary for circRNA_025636 (hsa_circ_0025636).	200
Figure 6-43 STRING network centred on RASSF8 created using Genecards.org.....	201

Figure 7-1 NCCP Chemotherapy Regimen for Carboplatin / Paclitaxel	214
Figure 7-2 COV362 cells showing increased cell death with escalating carboplatin concentrations	217
Figure 7-3 Kuramochi cells showing response to escalating carboplatin concentrations.....	217
Figure 7-4 OVSAHO cells showing response to escalating carboplatin concentrations	218
Figure 7-5 Dose–response curves for COV362 across four treatment models	222
Figure 7-6 Dose–response curves for OVSAHO across four treatment models	223
Figure 7-7 Dose–response curves for KURAMOCHI across four treatment models	224
Figure 7-8 OD450 readings at varying cell densities and time points for COV362 cells	229
Figure 7-9 OD450 readings at varying cell densities and time points for KURAMOCHI cells	230
Figure 7-10 OD450 readings at varying cell densities and time points for OVSAHO cells	231
Figure 7-11 Assessment of chemotherapy resistance in COV362 using CCK8 assay.....	233
Figure 7-12 Assessment of chemotherapy resistance in OVSAHO using CCK8 assay.....	234
Figure 7-13 Assessment of chemotherapy resistance in KURAMOCHI using CCK8 assay	235
Figure 7-14 BrdU viability of COV362 parental and resistant lines.....	236
Figure 7-15 Cellular proliferation of OVSAHO parental and resistant lines.....	237
Figure 7-16 BrdU viability of Kuramochi parental and resistant lines	238
Figure 7-17 Assessing PARPi sensitivity in chemotherapy-resistant models	239
Figure 7-18 Expression of stemness markers in chemotherapy-resistant OC models.	241
Figure 7-19 Expression of stemness markers in PARPi-resistant OC models.	242
Figure 7-20 Expression of influx and efflux markers in treatment-resistant OC cell lines.	243
Figure 8-1 Heatmap of circRNA expression chemo-resistant vs. UT	254
Figure 8-2 Heatmap of circRNA expression PARPi-resistant vs. UT.....	255
Figure 8-3 Scatter plot of circRNA expression in chemo-resistant vs. UT	257
Figure 8-4 Scatter plot of circRNA expression in PARPi-resistant vs. UT	258
Figure 8-5 circRNA chromosomal distribution in Chemo-resistant vs. UT	260
Figure 8-6 circRNA chromosomal distribution in PARPi-resistant vs. UT	261
Figure 8-7 Gene Ontology enrichment of host genes of the circRNA in chemo-resistant vs. UT.....	263
Figure 8-8 GO BP enrichment for circRNA host genes in chemo-resistant vs. UT	264
Figure 8-9 GO CC enrichment (fold enrichment) in chemo-resistant vs. UT	265
Figure 8-10 GO CC enrichment (GeneRatio) in chemo-resistant vs. UT	266
Figure 8-11 GO MF enrichment (fold enrichment) in chemo-resistant vs. UT	267
Figure 8-12 GO MF enrichment (GeneRatio) in chemo-resistant vs. UT	268
Figure 8-13 GO enrichment of circRNA host genes in PARPi-resistant vs. UT	270
Figure 8-14 GO BP enrichment in PARPi-resistant vs. UT	271
Figure 8-15 GO CC enrichment (fold enrichment) in PARPi-resistant vs. UT.....	272
Figure 8-16 GO CC enrichment (GeneRatio) in PARPi-resistant vs. UT.....	273
Figure 8-17 GO MF enrichment (fold enrichment) in PARPi-resistant vs. UT.....	274
Figure 8-18 GO MF enrichment (GeneRatio) in PARPi-resistant vs. UT.....	275
Figure 8-19 KEGG enrichment of upregulated circRNA host genes, chemo-resistant vs. UT.....	276
Figure 8-20 KEGG enrichment of upregulated circRNA, PARPi-resistant vs. UT.....	277
Figure 8-21 Venn diagram showing circRNAs upregulated in resistant vs. UT cells.....	279
Figure 8-22 Visualisation of endpoint PCR products in (chemo-resistant COV362 model.....	280
Figure 8-23 Visualisation of endpoint PCR products in , PARPi-resistant COV362 model.....	281
Figure 8-24 qPCR validation of circRNA expression in COV362 resistant models	282

Tables

Table 1-1 Clinical and Genetic Characteristics of Epithelial OC Subtypes.	1
Table 1-2 Five-Year Cause-Specific Survival (%) for EOC / HGSOC	2
Table 1-3 Clinical trials of PARP inhibitors as maintenance in the front-line setting	5
Table 1-4 PFS and OS results from the Ariel 3 Trial	5
Table 1-5 Clinical trials of PARP inhibitors as maintenance in the recurrent setting	6
Table 1-6 Frequency of BRCA Mutations in newly diagnosed HGSOC	13
Table 1-7 circRNA as Biomarkers in OC	25
Table 1-8 circRNA and Drug Resistance	26
Table 1-9 CDR1 in Cancer	28
Table 2-1 qPCR conditions.	43
Table 3-1 Characteristics of Cohort Referred for Testing	59
Table 3-2 Germline and Somatic <i>BRCA1/2</i> CAV prevalence.	62
Table 3-3 Germline and Somatic <i>BRCA1/2</i> CAV prevalence for HGSOC subgroup	63
Table 3-4 Most frequently reported g <i>BRCA1/2</i> CAV	65
Table 3-5 PVs identified in <i>BRCA1/2</i> : mutation type, and genomic details.	69
Table 3-6 Cost Analysis and Detection Sensitivity of Integrated and Reflex Testing Models	71
Table 3-7 Clinicopathological Characteristics of the Young-Onset OC Cohort	73
Table 3-8 Summary of Overall Survival (OS) by Subtype	77
Table 3-9 Summary of OS by Stage	78
Table 3-10 Summary of OS by Grade	79
Table 3-11 YOC: Genetic Testing and Variant Detection	81
Table 3-12 Correlation between OC subtype and variable	82
Table 4-1 Demographic Characteristics of the CAMPaIGn Pilot Cohort (n = 70)	103
Table 4-2 Referral and Genetic Testing Characteristics	103
Table 4-3 Distribution of Pathogenic Variants	104
Table 4-4 Cancer-Related Characteristics	105
Table 4-5 Family History and Cascade Testing	106
Table 4-6 Respondent demographics from the PPI qualitative survey	108
Table 4-7 Engagement and communication preferences	108
Table 4-8 PPI governance preferences for a national registry	111
Table 5-1 Upregulated miRNA categories of interest across cell lines	120
Table 5-2 OS for selected miRNA from KM Plotter	122
Table 5-3 Genes Associated with selected miRNA as evaluated in TargetScan	123
Table 5-4 Association of gene expression with tumour stage and survival analysed in UALCAN.	124
Table 5-5 Statistical significance of gene related protein expression between normal and OC.	126
Table 5-6 Summary of the most significant identified genes, related cell line and BRCA status.	127
Table 5-7 miRNA-associated genes literature review	129
Table 5-8 Summary of genes comparing protein expression between normal and tumour tissue	134
Table 5-9 Significance of miRNA expression of BRCA mutant cell lines compared to the wildtype	143
Table 6-1 Cell Line Selection	154
Table 6-2 <i>BRCA1</i> and <i>BRCA2</i> Mutation Status in OC Cell Lines	160
Table 6-3 Top 5 Upregulated circRNA KURAMOCHI vs. OVSAHO	185
Table 6-4 Top 5 Upregulated circRNA COV362 vs. OVSAHO	185

Table 6-5 Top 6 Common upregulated circRNAs	186
Table 6-6 Left and right primers for selected upregulated circRNA	189
Table 6-7 circRNAs selected for validation across <i>BRCA</i> statuses	192
Table 6-8 CircRNAs for Further Validation After Primer Optimization and Validation	194
Table 6-9 OC Cell Line Panel and <i>BRCA</i> Status	196
Table 6-10 Cell lines used in circRNA in OC literature above.....	203
Table 7-1 Clinical vs. laboratory resistance models: Key Features	213
Table 7-2 Dose Optimisation for Treatment Summary	216
Table 7-3 LogIC ₅₀ Values Across OC Cell Lines, Treatments, and Timepoints	221
Table 7-4 Dose Optimisation for Treatment Summary	225
Table 7-5 Summary of Resistance Induction Across OC Cell Lines and Treatment	227
Table 8-1 Top three upregulated circRNAs in chemo-resistant vs untreated	278
Table 8-2 Top three upregulated circRNAs in PARPi-resistant vs untreated	279
Table 8-3 Divergent primer pairs for selected circRNAs	280

Abbreviations

AJCC	American Joint Committee on cancer
Anti-VEGF	Anti-Vascular Endothelial Growth Factor
ASCO	American Society of Clinical Oncology
BER	Base Excision Repair
BRCA1	Breast Cancer Gene 1
BRCA2	Breast Cancer Gene 2
CCNE1	Cyclin E1
CCOC	Clear Cell OC
circRNA	Circular RNA
CTR1	Copper Transporter 1
DDR	DNA Damage Repair
DNA	Deoxyribonucleic Acid
DSB	Double Stranded Breaks
HER	Electronic Health Records
HRD	Homologous Recombination Deficiency
HRR	Homologous Recombination Repair
EMA	European Medicines Agency
ENOC	Endometrioid OC
EOC	Epithelial OC
ER	Estrogen Receptor
ESMO	European Society Medical Oncology
FDA	U.S. Food and Drug Administration
FIGO	International Federation of Gynaecology and Obstetrics
gBRCA	Germline BRCA
GO	Gene Ontology

HGSOC	High Grade Serous OC
HR	Homologous Recombination
HRD	Homologous Recombination Deficient
HSE	Health Services Executive
ITT	Intention To Treat
KEGG	Kyoto Encyclopedia of Genes and Genomes
LGSOC	Low Grade Serous OC
MMR	Mismatch Repair
MOC	Mucinous OC
NCCN	National Cancer Comprehensive Network
NCCP	National Cancer Control Program
NER	Nucleotide Excision Repair
NHEJ	Non-Homologous End Joining
PV	Pathogenic Variant
OC	Ovarian Cancer
ORR	Objective Response Rates
OS	Overall Survival
PARPi	Poly-(ADP)-ribose Polymerase Inhibitors
PFS	Progression Free Survival
PFS2	Time to second disease progression
PR	Progesterone Receptor
RNA	Ribonucleic acid
SACT	Systemic Anti-Cancer Therapy
sBRCA	Somatic BRCA
SD	Stable Disease
smRNA	Small RNA

SSA	Single Stranded Annealing
SSB	Single Strand Breaks
TAH BSO	Total Abdominal Hysterectomy and Bilateral Salpingo-Oophorectomy
tBRCA	Tumour BRCA
TGGA	The Cancer Genome Atlas

Units

°C	degrees Celsius
bp	base pairs
Da	daltons
g	grams
h	hour(s)
kB	kilobases
kg	kilograms
L	litre(s)
hr	hour
M	molar
mg	milligram
min	minutes
mL	millilitre
mM	millimolar
mm	millimetre
n	number (sample size)
pg	picogram
rpm	revolutions per minute
s	second(s)
U	unit(s)
v/v	volume per volume
µg	microgram
µL	microlitre
µM	Micromolar

Publications, Presentations and Awards

Original Research Publications

Flynn, J., Baird, A.M., Breen, E., Carty, M., **McNevin, CS.**, McDermott, L., Kenny, E., Davis Coakley, J., Ryan, T., Doherty, DG., Sheils, O. Multi-omic analysis of PBMCs in sepsis reveals widespread cytotoxic dysfunction and an increased population of CD69 expressing naïve CD4+ T cells. *Frontiers in Immunology*, 2025; 16. Accessible from <https://doi.org/10.3389/fimmu.2025.1667186>

McNevin, CS., Mohammed Nur, M., O'Brien, C., McGovern, B., McFadden, J., Baird, AM., Cadoo, KA., Barr, MP., Gray, SG., Keogh, AP., Sheils, O., Sutton, LA., Flanagan, S., Mucci, LA., Stopsack, KH., Finn, SP. Prevalence of mismatch repair deficiency in a large prospective prostate cancer cohort. *Clinical Cancer Research*, 2025; 31(3). Accessible from <https://doi.org/10.1158/1078-0432.CCR-24-1210>

McNevin, CS., Cadoo, K., Baird, A.M., Finn, S.P., McDermott, R. PARP Inhibitors in Advanced Prostate Cancer in Tumors with DNA Damage Signatures. *Cancers (Basel)* 2022; 14(19):4751. doi: 10.3390/cancers14194751. Accessible from <https://pubmed.ncbi.nlm.nih.gov/36230674/>

Lim, M.C.J., Baird, A.M., Greene, J., **McNevin, C.**, Ronan, K., Podlesniy, P., Sheils, O., Gray, S.G., McDermott, R.S., Finn, S.P. hsa_circ_0001275 Is One of a Number of circRNAs Dysregulated in Enzalutamide Resistant Prostate Cancer and Confers Enzalutamide Resistance In Vitro. *Cancers (Basel)* 2021; 13(24):6383. Accessible from <https://pubmed.ncbi.nlm.nih.gov/34945002/>

McNevin, CS., Cadoo, K., Baird, A.M., Murchan, P., Sheils, O., McDermott, R., Finn, S. Pathogenic *BRCA* Variants as Biomarkers for Risk in Prostate Cancer. *Cancers (Basel)* 2021; 13(22):5697. Accessible from <https://pubmed.ncbi.nlm.nih.gov/34830851/>

Muchan, P., O'Brien, C., O'Connell, S., **McNevin, CS.**, Baird, A., Sheils, O., O'Broin, P., Finn, S.P. Deep Learning of Histopathological Features for the Prediction of Tumour Molecular Genetics. *Diagnostics* 2021; 11(8):1406. Accessible from <https://pubmed.ncbi.nlm.nih.gov/34441338/>

Green, J., Baird, A., Lim, M., Flynn, J., **McNevin, CS.**, Brady, L., Sheils, O., Gray, S.G., McDermott, R., Finn, S.P. Differential CircRNA Expression Signatures May Serve as Potential Novel Biomarkers in Prostate Cancer. *Frontiers in Cells and Developmental Biology* 2021; 9:605686. Accessible from <https://pubmed.ncbi.nlm.nih.gov/33718350/>

McNevin, CS., Baird, A., McDermott, R., Finn, S.P. Diagnostic Strategies for Treatment Selection in Advanced Prostate Cancer. *Diagnostics* 2021; 11(2):345. Accessible from <https://pubmed.ncbi.nlm.nih.gov/33669657/>

Published Conference Abstracts

McNevin, C., Murphy, K., Murchan, P., Hennessy, A., Fitzpatrick, O., Dillon, R., Hutch, R., O'Toole, S., Smyth, P., Atkinson, E., Baird, A.-M., Gray, S., Doyle, B., McDevitt, T., Coleman, N., Thodi, G., Kalachand, R. D., Cadoo, K., O'Brien, C., Finn, S., O'Brien, C. 2367P *Modelling the Cost of BRCA Testing Strategies in Ovarian Cancer: Integrated vs Reflex Testing*. (Abstract). European Society for Medical Oncology (ESMO) Congress 2025, Berlin, October 17–21, 2025. Forthcoming abstract publication in *Annals of Oncology*.

Fitzpatrick, O., Khan, RM., **McNevin, C.**, McMahon, D., O'Kane, GM., McCarthy, M. 1553P *Call to Action! Disparities across healthcare systems*. (Abstract). *Annals of Oncology*. 35(Suppl 2), 2024. Accessible from: <https://doi.org/10.1016/j.annonc.2024.08.1615>

McNevin, CS., Dillon, R., Hollingsworth, S., Lewis, F., Hurley, S., Norris, L. A., Ward, M., Donachie, V., Maguire, P. J., Alken, S., Coleman, N., Kamran, W., Abu Saadeh, F., O'Gorman, C., O'Donnell, D., D'Arcy, T., O'Leary, J., Finn, S., O'Toole, S. A., Cadoo, K. 1210P *Genetics and Survival Outcomes in Young-Onset Ovarian Cancer: Insights From an 18-Year Tertiary Referral Center Study*. (Abstract). *International Journal of Gynecologic Cancer* 2025; 35(2). Accessible from <https://doi.org/10.1016/j.ijgc.2024.100350>

McNevin, CS., Murphy, K., Murchan, P., Hennessy, A., Fitzpatrick, O., O'Shaughnessy, M., O'Toole, S., Smyth, P., Atkinson, E., Thordi, G., Baird, A.-M., Gray, S., O'Brien, C., Doyle, B., Coleman, N., Finn, S., Kalachand, R.D., Cadoo, K.A. 1222P *Prevalence of BRCA1/2 Variants in Ovarian Cancer Cohort: Outcomes from a Nationwide Testing Program*. (Abstract). *International Journal of Gynecological Cancer*. 35(2). Accessible from <https://doi.org/10.1016/j.ijgc.2024.101151>

O'Reilly, D., Dowling, G., Marks, S., Barry, S., Moloney, C., Moran, L., Kieran, R., Cluxton, C., Kirwan, L., Deac, O., **McNevin, C.**, Hadi, D., Dillon, R., Reilly, A., Barr, M., Cuffe, S., Toomey, S., Hennessy, B., Naidoo, J. P2.11A.04 *Exhaled Breath Biomarkers of Response to Immunotherapy in Advanced Non-Small Lung Cancer*. (Abstract). *Journal of Thoracic Oncology* 2024; 19(10). Accessible from [https://www.jto.org/article/S1556-0864\(24\)01328-5/abstract](https://www.jto.org/article/S1556-0864(24)01328-5/abstract)

Lewis, F., Ward, M., O'Gorman, C., Henderson, B., Hurley, S., Beirne, J., O'Driscoll, L., **McNevin, C.**, Kelly, T., Gately, K., Oner, E., Saini, V., Norris, L., Kanjuga, M., Martin, C., Maguire, P., Cadoo, K., McManus, G., O'Leary, J., O'Toole, S. *Longitudinal Detection of Circulating Tumour Cells in Serous and Non-Serous Epithelial Ovarian Carcinomas*. (Abstract). *International Journal of Gynecologic Cancer* 2024; 34. Accessible from https://ijgc.bmj.com/content/34/Suppl_1/A425.1

McNevin, C.S., Mohammed Nur, M., O'Brien, C., McGovern, B., McFadden, J., Baird, A.M., Cadoo, K.A., Barr, M.P., Gray, S.G., Keogh, A.P., Sheils, O., Sutton, L.A., Flanagan, S., Mucci, L.A., Stopsack, K.H., Finn, S.P. *Prevalence of Mismatch Repair Deficiency in a Large Prospective Prostate Cancer Cohort*. (Abstract). *Journal of Clinical Oncology* 2023;

41(16_suppl):5031-5031. Accessible from
https://ascopubs.org/doi/10.1200/JCO.2023.41.16_suppl.5031

Continued...

Harvey, H., Amberger-Murphy, V., Ballot, J., O'Grady, M., O'Hare, D., Lawler, G., Bennette, E., Connolly, M., **McNevin, C.**, Noone, E., Kelly, M., Bazin, A., Kearns, K., Mulroe, E., McDermott, R., O'Reilly, S. Impact of Conti Ransomware Attack on Cancer Trials Ireland Sites. (Abstract). *Journal of Clinical Oncology* 2022; 40(16_suppl). Accessible from https://ascopubs.org/doi/abs/10.1200/JCO.2022.40.16_suppl.e13614

Poster Presentations at International Conferences

McNevin, C., Murphy, K., Murchan, P., Hennessy, A., Fitzpatrick, O., Dillon, R., Hutch, R., O'Toole, S., Smyth, P., Atkinson, E., Baird, A.-M., Gray, S., Doyle, B., McDevitt, T., Coleman, N., Thodi, G., Kalachand, R. D., Cadoo, K., O'Brien, C., Finn, S. *Modelling the Cost of BRCA Testing Strategies in Ovarian Cancer: Integrated vs Reflex Testing. (Abstract).* **European Society for Medical Oncology (ESMO)**; *ESMO Congress 2025*, Berlin, October 17–21, 2025.

McNevin, C., Dillon, R., Hollingsworth, S., Lewis, F., Hurley, S., Norris, L. A., Ward, M., Donachie, V., Maguire, P. J., Alken, S., Coleman, N., Kamran, W., Abu Saadeh, F., O'Gorman, C., O'Donnell, D., D'Arcy, T., O'Leary, J., Finn, S., O'Toole, S. A., Cadoo, K. *Genetics and Survival Outcomes in Young-Onset Ovarian Cancer: Insights From an 18-Year Tertiary Referral Center Study.* Presented as a paper poster at The **European Society of Gynaecological Oncology (ESGO)**; Rome, February 20-23, 2025.

McNevin, C., Murphy, K., Dillon, R., Murchan, P., Hennessy, A., Fitzpatrick, O., O'Shaughnessy, M., O'Toole, S. A., Thordi, G., Smyth, P., O'Brien, C., Atkinson, E., Baird, A.-M., Gray, S., Doyle, B., Coleman, N., Finn, S., Kalachand, R. D., Cadoo, K. *Prevalence of BRCA1/2 in an Ovarian Cancer Cohort: Outcomes From a Nationwide Testing Program.* Presented as an ePoster at The **European Society of Gynaecological Oncology (ESGO)**; Rome, February 20-23, 2025.

O'Reilly, D., Dowling, G., Marks, S., Barry, S., Moloney, C., Moran, L., Kieran, R., Cluxton, C., Kirwan, L., Deac, O., **McNevin, C.**, Hadi, D., Dillon, R., Reilly, A., Barr, M., Cuffe, S., Toomey, S., Hennessy, B., Naidoo, J. *P2.11A.04 Exhaled Breath Biomarkers of Response to Immunotherapy in Advanced Non-Small Lung Cancer.* Presented at the **World Conference on Lung Cancer (WCLC)** 6th – 9th September 2024

Fitzpatrick, O., Khan, RM., **McNevin, C.**, McMahon, D., O'Kane, GM., McCarthy, M. *Call to Action! Disparities across healthcare systems.* Presented at the **European Society Medical Oncology (ESMO)**; Barcelona 7th – 10th Sept 2024

O'Reilly, D., Dowling, G., Marks, S., Barry, S., Moloney, C., Moran, L., Kieran, R., Cluxton, C., Kirwan, L., Deac, O., **McNevin, C.**, Hadi, D., Dillon, R., Reilly, A., Barr, M., Cuffe, S., Toomey, S., Hennessy, B., Naidoo, J. *Biomarkers of response to immunotherapy in advanced non-small lung cancer: Preliminary data from breath & blood cfDNA analysis.* Presented at **World Conference on Lung Cancer (WCLC)**; San Diego, September 7th – 10th, 2024

Flynn, J., Baird, AM., Breen, E., **McNevin, C.**, Mcdermott, L., Kenny, E., Kowalczyk, E., Coakley, JD., Ryan, T., Doherty, DG., Sheils, O. *Multi-omic single cell analysis reveals immune cell perturbations in sepsis.* Presented at **European Conference of Immunology (ECI)**; Dublin, 1st – 4th September 2024

McNevin, C., Lewis, F., O'Gorman, C., Abu Saadeh, F., O'Leary, J., Norris, L., Ward, M., J Maguire, P., Alken, S., Hurley, S., Donachie, V., O'Toole, S., Cadoo, K. *Clinical and Histopathologic Analysis of Young Onset Ovarian Cancer: A Tertiary Referral Centre*

Experience Over 18 Years. Presented at The **European Society Gynaecological Oncology (ESGO)**; Barcelona 7th – 10th March 2024

Continued...

Lewis, F., Ward, M., O’Gorman, C., Henderson, B., Hurley, S., Beirne, J. P., O’Driscoll, L., **McNevin, C.**, Kelly, T., Gately, K., Oner, E., Saini, V., Norris, L., Kanjuga, M., Martin, C. M., Maguire, P. J., Cadoo, K., McManus, G., O’Leary, JJ, O’Toole, S. Longitudinal detection of circulating tumour cells in serous and non-serous epithelial ovarian carcinomas. **European Society Gynaecological Oncology (ESGO)**. Barcelona, 7th – 10th March 2024.

McNevin, CS., Mohammed Nur, M., O’Brien, C., McGovern, B., McFadden, J., Baird, AM., Cadoo, KA., Barr, MP., Gray, SG., Keogh, AP., Sheils, O., Sutton, LA., Flanagan, S., Mucci, LA., Stopsack, KH., Finn, SP., *Frequency of Mismatch Repair Deficiency in Prostate Cancer: Experience from the Harvard Prostate Cohorts.* Presented at The **American Society of Clinical Oncology (ASCO)**; Chicago 2nd – 6th June 2023

Harvey H, Amberger-Murphy V, Ballot J, O’Grady M, O’Hare D, Lawler G, Bennette E, Connolly M, **McNevin C**, Noone E, Kelly M, Bazin A, Kearns K, Mulroe E, McDermott R, O’Reilly S. Impact of Conti ransomware attack on cancer trials Ireland sites (Abstract). **American Society of Clinical Oncology (ASCO)**; Chicago 2nd – 6th June 2022

M.C.J. Lim, J.P. Greene, A. Baird, S. Gray, **C. McNevin**, R. McDermott, S.P. Finn: *1972P - Circular RNA is associated with enzalutamide resistant prostate cancer.* Presented at **The European Society of Medical Oncology (ESMO)**; Virtual Meeting, 17 September 2020

Poster Presentations at National Conferences

Dillon, R., **McNevin, C.S.**, O'Toole, S., Thordi, G., Smyth, P., Atkinson, E., Baird, A.M., Gray, S., O'Brien, C., Coleman, N., Finn, S., Cadoo, K. Prevalence of BRCA1/2 Variants in Breast Cancer Cohort: Outcomes from a Nationwide Testing Program. Presented at the **Irish Society of Human Genetics (ISHG)** Annual Meeting, Iontas Theatre, National University of Ireland Maynooth; 12 September 2025.

McNevin, C., Murphy, K., Dillon, R., Murchan, P., Hennessy, A., Fitzpatrick, O., O'Shaughnessy, M., O'Toole, S. A., Thordi, G., Smyth, P., O'Brien, C., Atkinson, E., Baird, A.-M., Gray, S., Doyle, B., Coleman, N., Finn, S., Kalachand, R. D., Cadoo, K. Prevalence of BRCA1/2 in an Ovarian Cancer Cohort: Outcomes From a Nationwide Testing Program. Presented at the National Education Day for Doctors in Training and the **RCPI Trainee Awards, Royal College of Physicians of Ireland**, Dublin; 23 May 2025.

Dillon, R., **McNevin, CS.**, Hollingsworth, S., Lewis, F., O'Gorman, C., Abu Saadeh, F., O'Leary, J., Norris, L., Ward, M., J Maguire, P., Alken, S., Hurley, S., Donachie, V., O'Toole, S., Cadoo, K. *Clinical and Genetics and Survival Outcomes in Young-Onset Ovarian Cancer: Insights from an 18-Year Tertiary Referral Center Study*. Presented at the Presented at an **Irish Society of Medical Oncology Meeting (ISMO)**; RCSI, Dublin 1, 31st Jan 2025

Hollingsworth, S., **McNevin, CS.**, Dillon, R., Lewis, F., O'Gorman, C., Abu Saadeh, F., O'Leary, J., Norris, L., Ward, M., J Maguire, P., Alken, S., Hurley, S., Donachie, V., O'Toole, S., Cadoo, K. *Clinical and Histopathologic Analysis of Young Onset Ovarian Cancer: A Tertiary Referral Centre Experience Over 18 Years*. Presented at the **Junior Obstetrics and Gynaecology Society (JOGS)**. Dublin 2, 29th Nov 2024

McNevin, CS., Murphy, K., Murchan, P., Hennessy, A., Fitzpatrick, O., O'Shaughnessy, O'Toole, S., Thordi, G., Smyth, P., Atkinson, E., Baird, AM., Gray, S., O'Brien, C., Doyle., Coleman, N., Finn, S., Deepa Kalachand, R., Cadoo, K. Prevalence of BRCA1/2 Variants in Ovarian Cancer: Outcomes from a Nationwide Testing Program. Presented at **Irish Society of Human Genetics (ISHG)**. Dublin 19th September, 2024.

Lewis, F., Ward, M., O'Gorman, C., Henderson, B., Hurley, S., Beirne, J. P., O'Driscoll, L., **McNevin, C.**, Kelly, T., Gately, K., Oner, E., Saini, V., Norris, L., Kanjuga, M., Martin, C. M., Maguire, P. J., Cadoo, K., McManus, G., O'Leary, JJ, O'Toole, S. Enumerating circulating tumour cells in serous and non-serous epithelial ovarian cancers. Presented at **Irish Society of Gynaecology Oncology (ISMO)**. Belfast 1st, Dec 2023.

Lewis, F., Ward, M., O'Gorman, C., Henderson, B., Hurley, S., Beirne, J. P., O'Driscoll, L., **McNevin, C.**, Kelly, T., Gately, K., Oner, E., Saini, V., Norris, L., Kanjuga, M., Martin, C. M., Kamran, W., Saadeh, F. A., Maguire, P. J., Cadoo, K., McManus, G., O'Toole, S., O'Leary, JJ. Dynamic enumeration of circulating tumour cells in ovarian cancer. Trinity St. James's Cancer Institute. Presented at **Trinity Translational Medicine Institute (TTMI)**, Dec 8 2023.

Oral Presentations

McNevin, C. *"Building a Registry for Patients with Cancer-Predisposing Genetic Alterations: Our Journey and Insights."* **Presented to a virtual audience at National Cancer Control Programme (NCCP), 11th April 2024.**

McNevin, C., Murphy, K., Murchan, P., Hennessy, A., Fitzpatrick, O., O'Shaughnessy, M., O'Toole, S., Smyth, P., Atkinson, E., Baird, AB., Gray, S., O'Brien, C., Doyle, B., Coleman, N., Finn, S., Deepa Kalachand, R., Cadoo, C. *Prevalence and Characteristics of BRCA1/2 Pathogenic Variants in an Irish Ovarian Cancer Cohort.* Presented at **NCHD Research Competition 2024**; St James Hospital, Dublin 8th, 16th May 2024

McNevin, C., Lewis, F., O'Gorman, C., Abu Saadeh, F., O'Leary, J., Norris, L., Ward, M., J Maguire, P., Alken, S., Hurley, S., Donachie, V., O'Toole, S., Cadoo, K. *Clinical And Histopathologic Analysis Of Young Onset Ovarian Cancer: A Tertiary Referral Centre Experience Over 18 Years.* Presented at The **Irish Society Medical Oncology (ISMO)**; The Mater Hospital, Dublin 3, 19th Jan 2024

McNevin, C., Lewis, F., O'Gorman, C., Abu Saadeh, F., O'Leary, J., Norris, L., Ward, M., J Maguire, P., Alken, S., Hurley, S., Donachie, V., O'Toole, S., Cadoo, K. *Clinical And Histopathologic Analysis Of Young Onset Ovarian Cancer: A Tertiary Referral Centre Experience Over 18 Years.* Presented at The **Irish Society Gynaecology Oncology (ISGO)**; Belfast, 1st Dec 2023

McNevin C, Mutaz Mohammed Nur, Brianán McGovern, Julie McFadden, Anne-Marie Baird, Karen Cadoo, Cathal O'Brien, Steven Gray, Martin P Barr, Lesley Ann Sutton, Lorelei Mucci, Orla Sheils, Konrad Stopsack, and Stephen P. Finn, *Frequency of Mismatch Repair Deficiency in Prostate Cancer: Experience from the Harvard Prostate Cohorts* at **Irish Society of Medical Oncology Meeting (ISMO)**; The Mater Hospital, Dublin 3, 27th Jan 2023

HONORS / AWARDS

The William Stokes Best Poster Award, Runner Up Best Clinical Poster Award 2025

Shortlisted for an RCPI Best Poster Award, RCPI, Dublin, May 2025

Irish Society of Medical Oncology (ISMO) Bursary Award for Excellent Poster 2025

'Genetics and Survival Outcomes in Young-Onset Ovarian Cancer: Insights from an 18-Year Tertiary Referral Center Study'. Awarded at Irish Society of Medical Oncology (ISMO) Meeting; RCSI, Dublin 2

Irish Society of Human Genetics (ISHG) Runner Up Best Clinical Poster Award 2024

Shortlisted for an ISHG Best Clinical Poster Award, Dublin, Sept 2024

Irish Society of Gynaecology Oncology (ISGO) Educational Bursary Award 2023

Awarded bursary to attend International Gynaecological Cancer Society (IGCS) Conference, Dublin 2024

Irish Society of Medical Oncology (ISMO) Bursary Award for Excellent Oral Presentation 2024

'Clinical and histopathologic analysis of Young Onset Ovarian Cancer: A tertiary referral centre experience over 18 years'. Awarded at Irish Society of Medical Oncology Meeting; Nelson St, Dublin 7, Jan 2024

Irish Society of Gynaecology Oncology (ISGO) Educational Bursary Award 2023

Awarded at bursary to attend European Society Gynaecology (ESGO) at ISGO Meeting; Belfast, Dec 2023

E - Journal Winner for Jan/Feb 2023

'A Tale of Tumours'; RCPI, Frederick House, 19 South Frederick Street, Dublin 2, Ireland

MEDIA MENTIONS

Highlighted in The Medical Independent, ISMO Bursary Awards 2024 Highlights

Featured in Mindo, 03rd March 2024. Recognized for receiving the ISMO Bursary Award at the ISMO Annual Conference 2024.

<https://www.medicalindependent.ie/in-the-news/conference/ismo-bursary-awards-2024/>

Highlighted in UroToday, ASCO 2023 Prostate Cancer Conference Highlights

Coverage of research presented at the ASCO 2023 conference, focusing on the prevalence of mismatch repair deficiency in prostate cancer patients.

<https://www.urotoday.com/conference-highlights/asco-2023/asco-2023-prostate-cancer/145000-asco-2023-prevalence-of-mismatch-repair-deficiency-in-a-large-prospective-prostate-cancer-cohort.html>

Table of Contents

1	Introduction.....	2
1.1	Ovarian Cancer.....	2
1.2	Demographics	2
1.3	Risk Factors.....	3
1.3.1	Genetic Risk Factors	3
1.3.2	Non Genetic Risk Factors	3
1.4	Clinical Presentation.....	4
1.5	Staging and Grade	4
1.6	Histological Subtypes	5
1.7	Prognosis	2
1.8	Therapeutic Paradigm	2
1.8.1	Surgery.....	3
1.8.2	Chemotherapy	3
1.8.3	PARPi	4
1.8.4	Overview of OC in the Irish Context.....	8
1.9	Homologous Recombination Repair	11
1.9.1	Background	11
1.9.2	DNA Damage Repair Pathways	11
1.9.3	HRD and OC.....	12
1.9.4	BRCA contributors to HRD in OC.....	12
1.9.5	Other HR-associated mutations in OC	13
1.10	miRNA and OC	14
1.10.1	miRNA dysregulation in OC.....	14
1.10.2	miRNA associated with tumour suppression in OC.....	15
1.10.3	miR-34a as a tumour suppressor in OC	15
1.10.4	miRNA associated with tumour promotion in OC	17
1.10.5	miR-200 Family as a tumour promoter in OC.....	17
1.10.6	miRNA associated with EMT in OC	17
1.10.7	miR-200 Family and their role in EMT in OC.....	18
1.10.8	miR-9 and its role in EMT in OC.....	19
1.11	circRNA and OC	20
1.11.1	Background	20
1.11.2	Biogenesis of circRNAs.....	21
1.11.3	Characteristics of circRNA.....	21
1.11.4	Clinical Application of circRNA in OC	22
1.11.5	circRNAs as Potential Biomarkers in EOC	22
1.11.6	circRNAs and Drug Resistance in OC	22
1.11.7	CircRNAs relating to BRCA	27
1.11.8	circRNA – miRNA Networks	27
1.12	Drug Resistant Mechanisms.....	29
1.12.1	Platinum resistance	29
1.12.2	Influx and efflux transporters	29
1.12.3	DNA Repair.....	29
1.12.4	Taxol resistance	30

1.12.5	P-glycoprotein efflux channels	30
1.12.6	Isoform Alteration	30
1.12.7	The PI3K/AKT pathway	30
1.12.8	Upregulation of enzymatic pathways.....	31
1.12.9	B-cell lymphoma 2 (Bcl-2) family.....	31
1.12.10	PARPi resistance	32
1.12.11	Restoration of homologous recombination repair	32
1.12.12	Restoration of replication fork stability	34
1.12.13	Drug target-related resistance	34
1.12.14	Prioritising alternative DNA repair pathways.....	34
1.12.15	Markers of Stemness.....	35
1.13	Aims and Objectives	36
1.13.1	General Aims	36
1.13.2	Hypothesis	36
1.13.3	Specific Objections	36
2	Materials and Methods	38
2.1	Epidemiology Analysis Methodology.....	38
2.1.1	Ethical Approval	38
2.1.2	Study of prevalence of BRCA CAVs in an Irish cohort.....	38
2.1.3	Study of clinco-pathological characteristics of young people diagnosed with OC in Ireland	38
2.1.4	BRCA1/2 CAVs Detection	38
2.1.5	Variant Classification	39
2.1.6	Statistical Analyses.....	39
2.2	In Silico Analysis Pipeline.....	40
2.2.1	KM Plotter	40
2.2.2	Target Scan.....	40
2.2.3	cBioportal.....	40
2.3	Preparation and handling of materials	41
2.3.1	miRNA validation	41
2.3.2	cDNA synthesis for miRNA.....	41
2.3.3	Real-Time qPCR.....	42
2.3.4	miRNA Statistical Analysis.....	43
2.4	Cell Line Authentication	44
2.4.1	STR Profiling	44
2.4.2	BRCA Status Verification	44
2.5	Cell Culture	45
2.5.1	Cell Culture Reagents.....	45
2.5.2	Cell line propagation.....	45
2.5.3	Cell line subculture.....	45
2.5.4	Cryopreservation	45
2.5.5	Agarose gel electrophoresis.....	45
2.5.6	Antimicrobial Use.....	46
2.5.7	Mycoplasma testing.....	46
2.5.8	Cell Counting.....	46
2.6	Developing and Characterising Resistant Cell Line Models	47
2.6.1	Drug Treatment.....	47
2.6.2	Developing a Dose for Resistance Model Creation	48

2.7	Cell Viability and Proliferation Assays	48
2.7.1	Resazurin assay	48
2.7.2	Cell Counting Kit (CCK-8) assay	48
2.7.3	Bromodeoxyuridine (BrdU) incorporation assay	49
2.8	Setting Up Clinical Model.....	49
2.9	RNA/DNA isolation and quantification	49
2.9.1	RNA Isolation	49
2.9.2	RNA Isolation using Qiagen miRNeasy kit	49
2.9.3	RNA Isolation using TRI Reagent	50
2.9.4	DNA Isolation.....	50
2.9.5	RNA / DNA Quantification	51
2.9.6	RNA quality assessment	51
2.10	PCR.....	51
2.10.1	cDNA synthesis for gene expression analysis	51
2.11	circRNA Analysis.....	52
2.11.1	Cell line Preparation for circRNA analysis	52
2.11.2	circRNA microarray.....	52
2.11.3	circRNA validation.....	53
2.11.4	circRNA Data Analysis	54
2.11.5	Functional Enrichment Analysis.....	54
3	<i>Demographic and Epidemiologic Landscape of OC in Ireland.....</i>	56
3.1	Introduction	56
3.1.1	Genetic Landscape of OC in Ireland	56
3.1.2	Characteristics and Challenges of OC in Young Women.....	56
3.2	Aims and Objectives	58
3.2.1	General Aims	58
3.2.2	Hypotheses.....	58
3.2.3	Objectives.....	58
3.3	Results.....	59
3.3.1	Nationwide Prevalence and Clinical Impact of <i>BRCA1/2</i> Mutations in OC.....	59
3.3.2	Patient Cohort Characteristics	59
3.3.3	Prevalence of Germline <i>BRCA1/2</i> Mutations	60
3.3.4	Characteristics of the <i>BRCA1/2</i> Population	64
3.3.5	Characteristics of <i>BRCA1/2</i> Variants	65
3.3.6	Economic Evaluation of <i>BRCA</i> Testing in Ovarian Cancer	71
3.3.7	Clinico-pathological and genetic characteristics of Young People with OC	72
3.3.8	Young People with OC Study Patient Cohort	72
3.3.9	Clinicopathological Features	72
3.3.10	Survival Outcomes for Young People with OC.....	75
3.3.11	Genetic Testing Results.....	80
3.3.12	Analysis of Risk Factors	82
3.4	Discussion	83
3.4.1	Prevalence of <i>BRCA1/2</i> PVs in a cohort of Irish OC patients	83
3.4.2	Survival Trends and Pathologic Features of OC in Young Women	86
4	<i>Designing and Launching a Pilot Registry for Cancer-Predisposing Genetic Mutations</i>	90

4.1	Introduction	90
4.2	Aims and Objectives	92
4.2.1	General Aims	92
4.2.2	Hypothesis	92
4.2.3	Objectives	92
4.3	Results	93
4.3.1	Conceptualising of the CAMPaIGn Study Registry	93
4.3.2	Information Gathering – Insights from Conferences and Networking	94
4.3.3	Identifying Gaps in Current Genetic and Cancer Registry Data	94
4.3.4	Research Existing Registries	94
4.3.5	Engagement with Stakeholders	95
4.3.6	Finalising the Registry Framework and Rationale	98
4.3.7	IT / Data Considerations	98
4.3.8	Consolidating the Conceptual Framework	99
4.4	Development of the Pilot Registry	100
4.4.1	Study Design	100
4.4.2	Launch of the Pilot Registry	102
4.5	Preliminary Data from the Pilot Registry	102
4.5.1	Cohort and Consent	102
4.5.2	Genetic Testing and Variant Spectrum	103
4.5.3	Disease Status and Index Cancer	104
4.5.4	Family History and Cascade Testing	105
4.5.5	Summary	106
4.6	Gathering Insights for the Registry's Development: PPI Survey	107
4.6.1	Respondent demographics	107
4.6.2	Engagement with the registry	108
4.6.3	Data sharing preferences	108
4.6.4	Registry priorities	110
4.6.5	Governance and oversight	110
4.6.6	Future applications	111
4.7	Discussion	112
5	<i>Expression Profiling of miRNA in Ovarian Cancer</i>	115
5.1	Introduction	115
5.2	Aims and Objectives	117
5.2.1	General Aims	117
5.2.2	Hypothesis	117
5.2.3	Objectives	117
5.3	Results	118
5.3.1	miRNA Identified from In Silico Analysis of OC	118
5.3.2	smRNA In Silico assessment	121
5.3.3	Comparison between findings and current literature	128
5.3.4	Prognostication of genes as reviewed in cBioportal	129
5.4	In Vitro Validation of selected miRNA	134
5.4.1	Selection of miRNA Targets and primer design	134
5.4.2	Optimisation of primers	134
5.4.3	Validation of selected targets	140

5.5	Discussion	144
5.6	Interpretation of Results	144
5.6.1	miR-652-3p	144
5.6.2	miR-3613	144
5.6.3	miR-27b-3p.....	144
5.6.4	miR_205-5p	145
5.6.5	miR_590-3p	146
5.6.6	miRNA and <i>BRCA</i>	148
5.7	Limitations	149
5.7.1	Limitations of In Silico Work	149
5.7.2	Limitations of Cell Line Experiments	149
5.7.3	Challenges in Identifying Clinically Relevant Targets	150
5.7.4	Potential Clinical Application	150
6	<i>Exploring the Relationship between BRCA and circRNA.....</i>	152
6.1	Introduction	152
6.2	Aims and Objectives	153
6.2.1	General Aims	153
6.2.2	Hypothesis	153
6.2.3	Objectives.....	153
6.3	Results	154
6.4	Cell Line Selection, Verification and Characterisation	154
6.4.1	Cell Line Selection	154
6.4.2	Cell Line Morphology	155
6.4.3	Mycoplasma testing	158
6.4.4	Mycoplasma Clearance	158
6.4.5	Cell Line Authentication	159
6.4.6	<i>BRCA</i> Characterisation and Verification.....	160
6.4.7	RNA Quality Check	161
6.5	Visualisation of circRNA Expression Patterns.....	162
6.5.1	Hierarchical Clustering Heatmaps	162
6.5.2	Scatter Plots	165
6.5.3	Volcano Plots.....	166
6.5.4	Gene Ontology Enrichment in <i>BRCA</i> Linked circRNAs	166
6.6	GO Enrichment in COV362 vs. OVSAHO.....	168
6.6.1	Biological Processes (BPs)	168
6.6.2	Cellular Components (CCs).....	169
6.7	Molecular Function (MFs).....	170
6.7.1	GO Enrichment in KURAMOCHI vs OVSAHO	171
6.7.2	GO Enrichment in COV362 vs KURAMOCHI	174
6.7.3	Pathways Enriched in <i>BRCA</i> Linked circRNAs.....	177
6.7.4	KEGG Pathway Enrichment in COV362 vs OVSAHO.....	178
6.7.5	KEGG Pathway Enrichment in KURAMOCHI vs OVSAHO	179
6.7.6	KEGG Pathway Enrichment in COV362 vs KURAMOCHI	180
6.7.7	Chromosomal Distribution of Differentially Expressed circRNAs	181
6.7.8	Differentially expressed circRNA profiling of OC cell lines	184
6.7.9	Validation of circRNAs in cell lines	187
6.7.10	Selection, Design, and Optimization of circRNAs	187

6.7.11	Primers Selected for BRCA mutant specific.....	190
6.7.12	Primers Selected for BRCA wildtype specific.....	193
6.7.13	Cell Line Validation of circRNAs Using RT-PCR	194
6.7.14	Evaluation of circRNA Patterns in a Panel of OC Cell Lines	196
6.7.15	Validation circRNA in clinical samples	198
6.7.16	Exploring circRNA 025636	200
6.8	Discussion	202
6.8.1	Cell Line Design Approach.....	202
6.8.2	Expression Patterns of BRCA-Linked circRNAs	203
6.8.3	Functional Landscapes of BRCA-Linked circRNAs	204
6.8.4	Biological Interpretation of BRCA-Linked KEGG Pathways.....	205
6.8.5	circRNAs as Biomarker of BRCA	206
6.8.6	circRNAs 0025636	208
6.8.7	Conclusion	208
7	The Development of Resistant OC Models: Lessons and Limitations	210
7.1	Introduction	210
7.2	Aims and Objectives	212
7.2.1	General Aims	212
7.2.2	Hypothesis	212
7.2.3	Objectives	212
7.3	Results	213
7.3.1	Strategies for Generating Resistance Models	213
9.1.1	Preliminary Data Guiding Development of Clinically Relevant Resistance Models	216
7.3.2	IC ₅₀ to Guide Development of Resistance Models	219
7.3.3	Transition to a Clinically Relevant Dosage Approach	225
7.3.4	Demonstrating Drug Resistance across cell lines	228
7.3.5	STR Profiling of resistant cell lines	239
7.3.6	Assessing PARPi Sensitivity in Chemotherapy Resistant Models	239
7.3.7	Stemness Profiling of Resistant OC Models	240
7.3.8	Influx and Efflux Marker Expression in Resistant OC Models	243
7.4	Discussion	244
7.4.1	Rationale and Limitations in Drug Dosing for Resistance Models	244
7.4.2	Cross-Resistance between Chemotherapy and PARPi	245
7.4.3	Stemness-Associated Adaptations in Resistance	246
7.4.4	Drug Transporter Dynamics in Resistant Models	246
7.4.5	Methodological Considerations in assays used to determine resistance	246
7.4.6	Challenges Encountered and Limitations	247
7.5	Future Work.....	249
8	circRNA profiling in Treatment Resistant OC	251
8.1	Introduction.....	251
8.2	Aims and Objectives	252
8.2.1	General Aims	252
8.2.2	Hypothesis	252
8.2.3	Objectives	252
8.3	Results	253
8.3.1	Visualisation of circRNA Expression Patterns in Resistant Models	253

8.3.2	Chromosomal Distribution of Resistance-Linked circRNAs	259
8.3.3	GO Enrichment in circRNAs UT vs Chemo-resistant.....	262
8.3.4	GO Enrichment in circRNAs UT vs PARPi-resistant.....	269
8.3.5	Pathways Enriched in treatment-resistant cells	276
8.3.6	Differential circRNA Profiles in Resistant Vs. Parental OC Cell Lines	277
8.3.7	Combined Resistance Overlap.....	279
8.3.8	Validation of circRNA Expression in Resistant Cell Lines.....	280
8.4	Discussion	282
9	<i>Discussion, Future Works and Conclusion</i>	287
9.1	Discussion	287
9.2	Summary of Contribution of Knowledge	290
9.3	Future Directions.....	292
9.4	Overall Conclusion	299
10	<i>References.....</i>	301
11	<i>Appendix.....</i>	327
	Appendix 1 : Custom designed Primer sequence for miRNA	327
	Appendix 2 : Case Report Form (CRF) for CAMPAIGN Registry.....	328
	Appendix 3 : Patient Information Leaflet for CAMPAIGN Registry	335
	Appendix 4 : Non-Significant Genes Identified in UALCAN TCGA Dataset Interrogation.....	341
	Appendix 5 : Non-Significant Genes Identified UALCAN Proteomic Expression Profile.....	342
	Appendix 6 : Search Strings Utilized for OC Gene Association Studies.....	343
	Appendix 7 : Summary of drug dosing concentrations and corresponding plasma levels for cell line treatment models.....	344
	Appendix 8 : Custom designed primer sequence for circRNAs	347
	Appendix 9 : Dosing schedule and escalation of Carboplatin, Paclitaxel, Olaparib, and combination doublet therapy across OC cell lines.....	348
	Appendix 10 : STR Profiling results	349
	Appendix 11 : Primers for circRNA resistance models	350

Summary

Ovarian cancer (OC) has the highest mortality rate among gynaecological malignancies. The most common subtype, high grade serous OC (HGSOC), frequently presents at an advanced stage due to the absence of effective screening programmes and the presence of vague symptoms, which often contribute to diagnostic delay. Although most women initially respond to treatment (a combination of surgery and systemic anti-cancer therapies), drug resistance inevitably develops, and the median survival following recurrence is approximately two years. Advances in the understanding of HGSOC biology have demonstrated that up to 50% of cases are driven by genetic factors, with the most frequent alterations occurring in *BRCA1* and *BRCA2*. Carriage of a pathogenic germline variant in *BRCA1* or *BRCA2* is a major risk factor for developing HGSOC, with lifetime risks estimated at 40–50% and 10–20%, respectively

Circular RNAs (circRNAs) have emerged as important molecules with prognostic, diagnostic, and therapeutic potential in OC. However, their assessment in OC with a focus on *BRCA* status is novel. To date, most studies have focused on cell lines, which may not accurately represent HGSOC. The potential role of circRNAs as predictive biomarkers of resistance to poly-(ADP)-ribose polymerase inhibitors (PARPi) has only been explored in limited preclinical models, which may not reflect the clinical setting. Consequently, the biological processes driving acquired resistance to these therapies across pathogenic germline *BRCA* variants remain poorly understood.

This project identified a lack of coordinated research and data infrastructure in this field. To address this, ethics approval was obtained to allow reporting of outputs from the Cancer Molecular Diagnostics (CMD) service, enabling the first nationwide prevalence study of *BRCA* mutations in OC in Ireland. A pilot registry was subsequently developed to assess a broader spectrum of cancer-predisposing alterations, report outputs and embed patient and public involvement (PPI).

In parallel, experimental work was undertaken. A model comprising three cell lines with distinct *BRCA* profiles was profiled for circRNA expression to investigate potential biomarkers of *BRCA* status in OC. From this analysis, candidate circRNAs of interest were identified and prioritised for further study. These candidates were subsequently validated in OC cell lines and in patient tumour samples, supporting their exploration as potential biomarkers of clinical relevance. Further, resistance models were established by exposing OC cell lines to chemotherapy and PARPi. These resistant lines were characterised, including assessment of stemness features and influx/efflux transporter activity. The most promising resistant model, COV362 (*BRCA1*-mutant), was prioritised for further analysis, and circRNA candidates associated with acquired resistance

were identified and validated in additional cell lines.

Together, this body of work combines pilot registry development, in silico data analyses, experimental modelling, and clinical validation to advance understanding of circRNA biology and resistance mechanisms in OC across *BRCA* subtypes.

Introduction

Chapter 1.0

1 Introduction

1.1 Ovarian Cancer

Ovarian cancer (OC) originates from the tissues of the ovary or fallopian tubes (**Figure 1-1**) and has the highest mortality rate among gynaecological malignancies (1). Despite initial responses to chemotherapy and surgery, 80% of patients relapse within three years (2). Standard treatment involves paclitaxel and carboplatin, often achieving clinical remission (3). Up to 50% of high-grade serous ovarian carcinomas (HGSOC) are homologous recombination deficient (HRD), with one-third due to *BRCA1/2* mutations (4-6). *BRCA1/2*-mutated OC is linked to better prognosis due to increased sensitivity to platinum-based chemotherapy (7). PARP inhibitors (PARPi) have shown efficacy in clinical trials (8-12), with *BRCA1/2* mutations predicting response and helping select the best candidates for treatment (13, 14). However, mechanisms driving resistance to these therapies remain poorly understood, and identifying these pathways is crucial for improving treatment stratification.

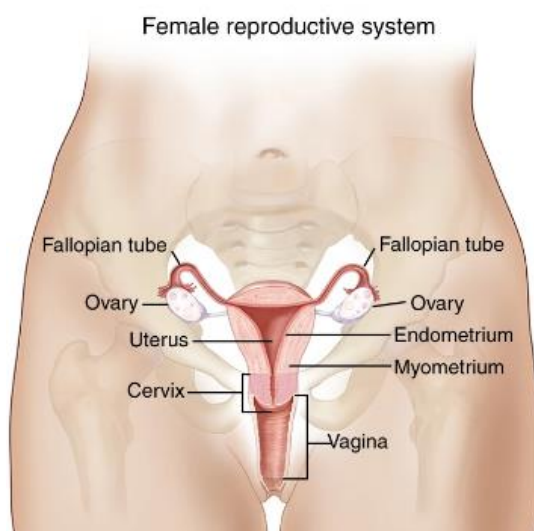


Figure 1-1 Female Reproductive System

1.2 Demographics

OC accounts for 2.5% of all malignancies among females but 5% of female cancer deaths (15), making OC the most lethal cancer in the female reproductive system. In Ireland alone, over 400 women are diagnosed with OC every year, with 272 women losing their lives due to the disease annually (16). Prevalence varies marginally across different ethnicities. Non-Hispanic white women appear to have the highest prevalence of OC (12.0 per 100,000), followed by Hispanic (10.3 per 100,000), non-Hispanic black (9.4 per 100,000), and Asian/Pacific Islander women (9.2 per 100,000) (15). OC predominantly affects postmenopausal women, although it can occur at any age. Notably, the histologic subtypes of the disease differ by age group. Germ cell tumours mainly occur in younger females with the median age at diagnosis being 16 to 20 years (17, 18). Borderline tumours (primarily mucinous and low grade serous) usually affect women aged between 20 and 40 (19), and women

with invasive epithelial OC (EOC), are mostly aged between 55 and 70 years (20, 21). According to the International Agency for Research on Cancer (IARC), the estimated global OC incidence for 2020 is 308,069 new cases and 193,811 deaths (22, 23). According to projections from Globocan 2022, the incidence of OC among women worldwide is expected to increase by over 55% by 2050, reaching a total of 503,448 diagnoses (24). Additionally, the annual mortality rate is anticipated to rise to 350,956, reflecting an increase of nearly 70% compared to 2022 figures.

1.3 Risk Factors

1.3.1 Genetic Risk Factors

Family history is a well-established risk factor for the development of EOC. Women with one affected first-degree relative is about three times as likely to develop EOC as women with no affected relatives (25). This risk increases if the relative was diagnosed below the age of 50 (20). Further a personal history of breast cancer is associated with an increased risk (26). Cases related to family history are commonly due to germline mutations in *BRCA1* and *BRCA2* genes (27). Inherited deleterious mutations in *BRCA1* and *BRCA2* are the major genetic risk factors for EOC (19). *BRCA1* mutation carriers have an estimated 40–50% risk of developing EOC by age 70, compared to 10–20% for *BRCA2* by the same age (28). EOC driven by *BRCA* mutations tend to occur at an earlier age than sporadic cancers, particularly in *BRCA1* mutation carriers, with a median age of diagnosis in the mid-40s (19). Strategies to negate the risk of EOC in *BRCA*-positive individuals include risk reducing salpingo-oophorectomy, which reduces the risk of EOC by 75% (29) and / or commencement of oral contraceptives, which reduce risk by about 50% among high risk women who have ever used them (15).

While the *BRCA* genes are the most prominent genes associated with EOC, many other genes associated with Homologous Recombination Repair (HRR), have been identified as increasing lifetime risk of developing the disease. *BRIP1*, *RAD1C* and *RAD1D* mutation carriers for instance, have estimated lifetime risks of developing OC of 5.8%, 5.2% and 12%, respectively (27). Cancer predisposing syndromes such as Lynch syndrome, a hereditary syndrome involving genes of DNA mismatch repair, is also a risk factor with individuals affected facing a lifetime risk of OC of about 6–8% (30), compared to 1.4% in the general population (19).

1.3.2 Non Genetic Risk Factors

OC has been linked with women having more ovulatory cycles in their lifetime (20). Therefore, risk factors include early menarche, older menopause, nulliparity and menopausal hormone therapy (19, 20). Conversely, factors which decrease hormone exposure including breastfeeding, use of the oral contraceptive pill and oophorectomy have been reported as having a protective effect (27). Other potential risk factors include obesity, diabetes, smoking, diet, physical exercise and usage of perineal talc (27). Chronic inflammation is also a risk factor for certain subtypes OC. Endometriosis is an established risk factor for patients with clear cell OC (CCOC) and high grade endometriosis tumours

(31, 32). Pelvic Inflammatory Disease (PID) may also increase the risk for OC (33), however, results vary across studies (34).

1.4 Clinical Presentation

Symptoms related to OC are unclear and hard to distinguish due to shared gastrointestinal, genitourinary, and gynaecological conditions, which can often lead to a long diagnostic course (2). Symptoms often include abdominal pain, bloating and early satiety (35). Gastrointestinal symptoms can be a presenting complaint such as change in bowel habit, constipation, or bowel obstruction (14). Urinary urgency and frequency can also be presenting symptoms. Concern should be raised if these symptoms are new and frequent and cannot be attributed to any known (previously identified) malignancy or cause (32). Screening for OC is currently not recommended by any society (32, 36) as the low prevalence of disease and lack of high-quality screening methods make it more likely to obtain false-positive results leading to unnecessary stress and result in unnecessary surgery.

1.5 Staging and Grade

OC is classified primarily as stages I to IV using the FIGO (International Federation of Gynaecology and Obstetrics) staging system based on tumour location (T), nodal involvement (N) and metastatic site (M), which was approved by the American Joint Committee on cancer (AJCC) (**Figure 1-2**) (32, 37). EOC can be further divided by histologic grading, which can be linked with prognosis; GX: Grade cannot be assessed, G1: Well differentiated, G2: Moderately differentiated, and G3: Poorly differentiated (19). Serous OC is either classified as low grade (most grade 1 serous tumours) or high grade (most grade 2 or 3 serous tumours) (32). Stage and grade of EOC dictates treatment course. For example, as per the National Cancer Comprehensive Network (NCCN) guidelines all patients with newly diagnosed HGSOc stage 1C or higher, or any patient with a grade 3 tumour regardless of stage, who have upfront debulking surgery, adjuvant chemotherapy is recommended if no contraindications.

	T	N	M
Stage I	T1	N0	M0
Stage IA	T1a	N0	M0
Stage IB	T1b	N0	M0
Stage IC	T1c	N0	M0
Stage II	T2	N0	M0
Stage IIA	T2a	N0	M0
Stage IIB	T2b	N0	M0
Stage IIIA1	T1/T2	N1	M0
Stage IIIA2	T3a	NX/N0/N1	M0
Stage IIIB	T3b	NX/N0/N1	M0
Stage IIIC	T3c	NX/N0/N1	M0
Stage IV	Any T	Any N	M1
Stage IVA	Any T	Any N	M1a
Stage IVB	Any T	Any N	M1b

Figure 1-2 AJCC Prognostic Groups

Taken from source (32)

1.6 Histological Subtypes

Traditionally, OC has been broadly classified according to cell type into epithelial and non-epithelial subtypes, the latter comprising germ cell, stromal cell, and small cell tumours (38, 39). EOC which accounts for over 90% of OCs, is itself a heterogeneous group that includes several histologically and molecularly distinct subtypes (15, 19). This heterogeneity has complicated the interpretation of histologic grade and prognostic outcomes in clinical studies (4, 42). To address this, EOC has been further categorised into two main groups based on a dualistic model of ovarian carcinogenesis: Type I and Type II tumours (32, 40, 41)(**Table 1-1**). Type I tumours include low-grade serous OC (LGSOC), CCOC, endometrioid OC (ENOC), and mucinous OC (MOC) (27). These neoplasms are genetically stable, typically present as large, slow-growing, unilateral cystic tumours, and are frequently confined to the ovary at diagnosis, resulting in relatively favourable prognoses (42, 43). They are often TP53 wild type, although some subtypes (e.g. MOC) may harbour TP53 mutations in a minority of cases. In contrast, Type II tumours - such as HGSOC, undifferentiated carcinomas, and carcinosarcomas - are characterised by near-universal TP53 mutations, marked genomic instability, rapid progression, and presentation at advanced stages, with poor overall survival (45, 278).

Epithelial (90%)							Reference
	Type II		Type I				
	HGSOc	Carcinosarcoma / undifferentiated	CCOC	ENOC	MOC	LGSOc	(42, 43)
Histologic subtype	70%	1.5%	10%	15%	3%	<5%	(42, 44)
Age at Diagnosis	55 - 70 yrs	> 60 yrs	55 yrs	50–60 yrs	20 – 40 yrs	20 – 40 yrs	(19-21, 45)
TP53 Status	Near-ubiquitous	Very common	20% of cases	17% of cases	64% (typically late-stage)	Rare	(46, 47)
Genetic Drivers	BRCA1 / BRCA2 HRR pathway genes (50%)		Inactivating mutations in <i>ARID1A</i> (50 – 60%), <i>PIK3CA</i> (20%) <i>PTEN</i> (10%), <i>T53</i> (17%), <i>MSI-H</i> (3%)	Inactivating mutations in <i>ARID1A</i> (30%), <i>PIK3CA</i> (20%) <i>PTEN</i> (15% to 20%) <i>MSI</i> (20% cases)		ER and PR** receptor common	(46, 48-51)
Tumour Marker	CA-125	CA-125	CA-125	CA-125	CA-125, CA 19-9, CEA	CA-125	(52, 53)
5 year OS	35%	20 – 30%	40 – 50%	80%	40 – 60%	60 – 80%	(42, 54-57)

Table 1-1 Clinical and Genetic Characteristics of Epithelial OC Subtypes.

ER refers to estrogen receptor and PR to progesterone receptor expression, both of which are commonly observed in low-grade serous OC. HRR denotes homologous recombination repair, and OS indicates overall survival.

1.7 Prognosis

A primary factor influencing the elevated mortality of HGSOC patients is the failure to diagnose the disease at an early, localized stage (27). The majority of EOC are diagnosed at late stage for which the five-year survival rate is low. For example, 51% will be diagnosed with stage III while 29% will be diagnosed with stage IV (15). There are currently no effective screening programmes in place for the early detection of OC and screening itself is currently not recommended by any society or medical body/association (27). The low prevalence of disease and lack of high-quality screening methods make it more likely to obtain false-positive results leading to unnecessary stress and resulted in unnecessary surgery (36). However, given the survival rate for women with EOC decreases as cancer progresses from stages I to IV (**Table 1-2**) (58), a lack of early detection is limiting ability to diagnose women at an earlier, more curable stage. It is important to note that patients carrying a *BRCA* mutation demonstrate a significant improvement in OS with the advent of PARPi therapy. At seven years, 67% of patients treated with Olaparib were all of whom had stage III and IV disease (59) were alive, a point further teased out in the following section.

Stage	Characterised	5 Year Survival (15) for all EOC	5 Year Survival (15) for all HGSOC
I	Tumour is limited to the ovary	89%	86%
II	Extension of tumour cells to pelvic organs	71%	71%
III	Tumour cells spreading to the pelvic and abdominal peritoneum, as well as retroperitoneal lymph nodes	41%	41%
IV	Distant metastasis	20%	26%

Table 1-2 Five-Year Cause-Specific Survival (%) for EOC / HGSOC

1.8 Therapeutic Paradigm

It is clear that EOC encompasses a wide range of histological and genetically distinct diseases. Despite this, historically the treatment for EOC has not appreciated this variability (60) and patients were treated uniformly. Surgery and chemotherapy have remained the backbone of treatment for all types of EOC for many decades. Development of targeted treatments such as PARPi and anti-vascular endothelial growth factor (Anti-VEGF) therapies have been a welcome addition to the treatment armamentarium in EOC, however these treatments hold specific distinctions that must be appreciated (61). Treatment decisions

are founded on many parameters including the time interval from last platinum therapy, histology, molecular profile, residual toxicities, and surgical resectability (3).

1.8.1 Surgery

Primary treatment for the majority of OCs consists of surgery, followed in most cases by systemic chemotherapy (62). 'Debulking surgery' and 'cytoreductive' are commutable terms to indicate surgical efforts aimed at removing the bulk of the tumour. For newly diagnosed patients, maximal effort should be made during a primary cytoreduction procedure to remove all gross disease since this offers superior survival outcomes (63). Studies have reported that for each 10% increase in maximal cytoreduction, a 5.5% increase in median survival time is evident (64, 65).

Debulking surgery is the initial treatment recommendation for patients with clinical stage II, III, or IV disease. For the majority of women, initial surgery should include total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH BSO), and further debulking as needed (63). There is also a role for interval debulking surgery post neoadjuvant treatment, which should be performed after 3–4 cycles of neoadjuvant chemotherapy for patients with either (a) response to chemotherapy or (b) stable disease (SD). For women who are keen to maintain fertility, a unilateral salpingo-oophorectomy (USO) and comprehensive surgical staging may be sufficient for select unilateral stage I tumours (stage 1A and 1C, not stage 1B) and/or EOC deemed to be low-risk (i.e., early-stage, grade 1 tumours; borderline tumours). For those with stage IB tumours who wish to maintain fertility, a BSO (preserving the uterus) and comprehensive surgical staging are recommended (62).

1.8.2 Chemotherapy

The primary treatment approach to EOC has not changed in over 30 years. A set of trials, completed in 1992, concluded that carboplatin, demonstrated comparable effectiveness to cisplatin in the treatment of OC but with a far more favourable toxicity profile (66). Since then, clinicians replaced cisplatin-based regimens (cisplatin / cyclophosphamide) with carboplatin-based regimens (carboplatin / cyclophosphamide) where possible (27). Around the same time, a new class of drugs, the taxanes, of which paclitaxel is the prototype, were developed (67). In 1996, a landmark clinical trial evaluated the effectiveness of cisplatin-paclitaxel combination therapy vs. cisplatin-cyclophosphamide. Results demonstrated that cisplatin-paclitaxel therapy was superior, significantly improving objective response rates (ORR), progression free survival (PFS) and OS (68). In the interest of averting the toxic side-effects of cisplatin, subsequent trials have confirmed similar results using carboplatin instead of cisplatin along with paclitaxel (69). The most recent clinical trial to assess various administration timings for paclitaxel and carboplatin was ICON8, which compared standard every 21-day dosing with weekly paclitaxel and every 21-day carboplatin with weekly dosing of both paclitaxel and carboplatin (3). No maintenance was used. The

PFS was similar in both studies, making 21-day paclitaxel and carboplatin the preferred option as it is less burden on the patient and the health system (70). ICON8 demonstrated the benchmark for survival in advanced EOC without selection by histology or molecular subtype, reporting a median PFS of 17.9 months (70). Many trials have compared this regime with other treatments including cyclophosphamide, docetaxel, topotecan, doxorubicin, epirubicin, gemcitabine, and melphalan (32) in doublet, triplet or quadruplet therapy combination, however, none have demonstrated superiority. Second line treatment can include liposomal doxorubicin, single agent taxol or topotecan (62).

1.8.3 PARPi

PARPi's are effective in EOC as demonstrated in a number of trials, spanning multiple PARPi, across *BRCA* status (8, 71, 72). However, much has changed since their introduction and several relevant questions such as their role in patients who are *BRCA* wildtype have been raised and remain unresolved in the post-PARPi era (73).

1.8.3.1 PARPi in the Upfront Setting

PARPi have demonstrated efficacy in the upfront setting in newly diagnosed EOC post-chemotherapy. The SOLO-1 trial first established olaparib as maintenance therapy, reporting a HR for PFS of 0.30 (95% CI, 0.23–0.41) and a median PFS of 49 months vs. 13.8 months for placebo (9). The benefit was greater in *BRCA2* than *BRCA1* carriers, leading to U.S. Food and Drug Administration (FDA) approval of olaparib in 2018 for patients with germline or somatic *BRCA*-mutated advanced EOC who had responded to platinum-based chemotherapy (74). At seven year follow up, 67.0% of patients treated with Olaparib vs. 46.5% of patients on placebo were alive (59).

As *BRCA* mutations represent only one marker of HRD, subsequent trials expanded eligibility. The PRIMA trial evaluated niraparib in both *BRCA* mutant and wildtype cohorts, showing a PFS benefit across all comers (HR 0.62; $P < 0.001$), with the greatest effect in the HRD subgroup (HR 0.43; $P < 0.001$) (75). This supported FDA approval of niraparib in the first-line setting for all patients; however, its international licensing has since shifted, with FDA approval for *BRCA* wildtype now revoked while EMA approval remains (76, 77).

The VELIA trial investigated veliparib given concurrently with chemotherapy followed by maintenance. Although the PFS benefit was modest (HR 0.68; 95% CI, 0.56–0.83), veliparib has not gained regulatory approval and is not recommended in current NCCN guidelines (62). Collectively, these trials confirm the role of PARPi as maintenance therapy following platinum response, while highlighting that combining PARPi with chemotherapy does not provide substantial additional benefit. The results of these trials are summarised below (**Table 1-3**).

Study	Phase	Population	Study Arm	Control Arm	Results
SOLO-1 NCT01844986	III	HGSOC/endometrioid (or FP/PPC) FIGO III-IV BRCAm CR/PR platinum-based chemotherapy	Olaparib 300 mg BID	Placebo	PFS NR vs. 13.8 m HR 0.30 (95% CI 0.23-0.41)
PRIMA NCT02655016	III	HGSOC/endometrioid (or FP/PPC) FIGO III-IV Regardless of BRCA status CR/PR platinum-based chemotherapy	Niraparib 300 mg daily	Placebo	ITT PFS 13.8 vs. 8.2 m HR 0.62 (95% CI 0.50-0.76) HRD PFS 21.9 vs. 10.4 m HR 0.43 (95% CI 0.31-0.59) BRCAmut 0.40 (95% CI, 0.27-0.62)
PAOLA-1 NCT02477644	III	HGSOC/endometrioid/other epithelial non-mucinous (or FP/PPC) FIGO IIIB, IIIC or IV gBRCAm/BRCAwt if HGS CR/PR platinum-based chemotherapy	Olaparib 300 mg BID + bevacizumab 15 mg/kg/3 wks	Placebo + Bevacizumab 15 mg/kg/3 wks	ITT PFS 22.1 vs. 16.6 m HR 0.59 (95% CI 0.49-0.72) BRCAmut HR 0.31 (95% CI 0.20-0.47)
VELIA NCT02470585	III	HGSOC OC (or FP/PPC) FIGO III-IV	Paclitaxel-carboplatin-veliparib (150 mg BID-2 weeks 400 mg BID) → veliparib	Paclitaxel-carboplatin-placebo → placebo	ITT PFS: 23.5 vs. 17.3 m HR 0.68 (95% CI 0.56-0.83) BRCAmut PFS: 34.7 vs. 22 m HR 0.44 (95% CI 0.28-0.68)

Table 1-3 Clinical trials of PARP inhibitors as maintenance in the front-line setting

Taken from source (78)

1.8.3.2 PARPi in the Relapsed / Maintenance Setting

In the phase III ARIEL3 trial evaluating relapsed EOC patients, rucaparib showed longer PFS in patients with BRCA-mutated disease (16.6 vs. 5.4 months, HR 0.23) and among HRD-positive and ITT cohorts (10.8 vs. 5.4 months, HR 0.37)(79),. However, while PFS outcomes were favourable across groups, OS data were less impressive compared with PFS2 in the HR population (Table 1.4).

	PFS2	OS
BRCA mutant:	26.1 vs. 18.4 months HR 0.67 (95% CI 0.48-0.94);	HR 0.83 (95% CI 0.58-1.19)
HRD	24.7 vs. 18.4 months HR 0.72 (95% CI 0.56-0.92)	HR 1.01 (95% CI 0.77-1.32)
ITT	20.6 vs. 16.3 months HR 0.70 (95% CI 0.58-0.85);	HR 1.00 (95% CI 0.81-1.22)

Table 1-4 PFS and OS results from the Ariel 3 Trial

Similarly, the phase III NOVA trial demonstrated that niraparib significantly improved PFS irrespective of germline *BRCA* mutation or HRD status, thereby extending the benefit of PARPi beyond *BRCA*-mutated cancers (14). Earlier trials further consolidated this evidence: Study 19 first demonstrated olaparib's benefit in recurrent *BRCA*-mutated OC (PFS 11.2 vs. 4.3 months, HR 0.18) (80), and SOLO-2 confirmed substantial prolongation of PFS in this population (19.1 vs. 5.5 months, HR 0.30)(81). Collectively, these trials established PARPi maintenance as a meaningful advance in the recurrent OC setting, offering durable disease control in both *BRCA*-mutated and non-mutated subgroups (Table 1-5).

Study	Phase	Population	Study Arm	Control Arm	Results
Study-19 NCT00753545	II	Recurrent HG (G2 or 3) OC/FP/PPC ≥2 platinum-based chemotherapy With an objective response to the platinum regimen	Olaparib 400 mg BID	Placebo	gBRCAm PFS 11.2 vs. 4.3 m HR 0.18 (95% CI, 0.10–0.31)
SOLO-2 NCT01874353	III	Recurrent OC/FP/PPC ≥2 platinum-based chemotherapy With an objective response to the platinum regimen BRCAm	Olaparib 300 mg BID	Placebo	PFS 19.1 vs. 5.5 m HR 0.30 (95% CI, 0.22–0.41)
NOVA NCT01847274	III	Recurrent HGSOC/FP/PPC ≥2 platinum-based chemotherapy Platinum sensitive (>6 months)	Niraparib 300 mg daily	Placebo	gBRCAm PFS: 21.0 vs. 5.5 m HR 0.27 (95% CI, 0.17–0.41) Non-gBRCA PFS 9.3 vs. 3.9 m HR 0.45 (95% CI, 0.34–0.61)
ARIEL-3 NCT01968213	III	Recurrent HGSOC/endometrioid (or FP/PPC) ≥2 platinum-based chemotherapy Platinum sensitive (>6 months) ≤1 non-platinum chemotherapy CR/PR platinum-based chemotherapy	Rucaparib 600 mg BID	Placebo	ITT PFS 10.8 m vs. 5.4 m HR 0.37 (95% CI, 0.30–0.45) BRCAm PFS 16.6 m vs. 5.4 m HR 0.23 (95% CI, 0.16–0.34)

Table 1-5 Clinical trials of PARP inhibitors as maintenance in the recurrent setting

Taken from source (78)

1.8.3.3 Targeted Therapies

The addition of bevacizumab, an anti-VEGF-targeted to chemotherapy following debulking surgery has become standard of care in many countries, supported by the PFS benefit observed in the randomised phase III GOG-0218 and ICON7 trials (13). Of note, this resulted in an improvement in PFS by approximately 4 months but no improvement in OS. This benefit in PFS was most pronounced in the high risk groups. Recent updates from the PAOLA-1 trial now suggest that the addition of bevacizumab to olaparib provides a clinically meaningful OS improvement for first-line patients with HRD-positive OC (10, 82). Maintenance

olaparib plus bevacizumab as a standard of care may enhance potential for cure for HRD disease in this setting.

1.8.4 Overview of OC in the Irish Context

Approximately 40,000 people in Ireland are diagnosed with invasive cancer (excluding non-melanoma skin cancer) every year (83). Among these, OC is particularly concerning ranked as the fourth most common cause of cancer deaths among women from 2013 to 2015, OC accounted for an average of 269 deaths annually (84). Ireland has a incidence rate of between 18.8 - 19.3 per 100,000 women (84, 85) compared to the EU average of 17.1 per 100,000 (85) (**Figure 1-3**). Projections indicate that by 2045, the incidence of OC in Ireland will increase by 67% to 80% (86).

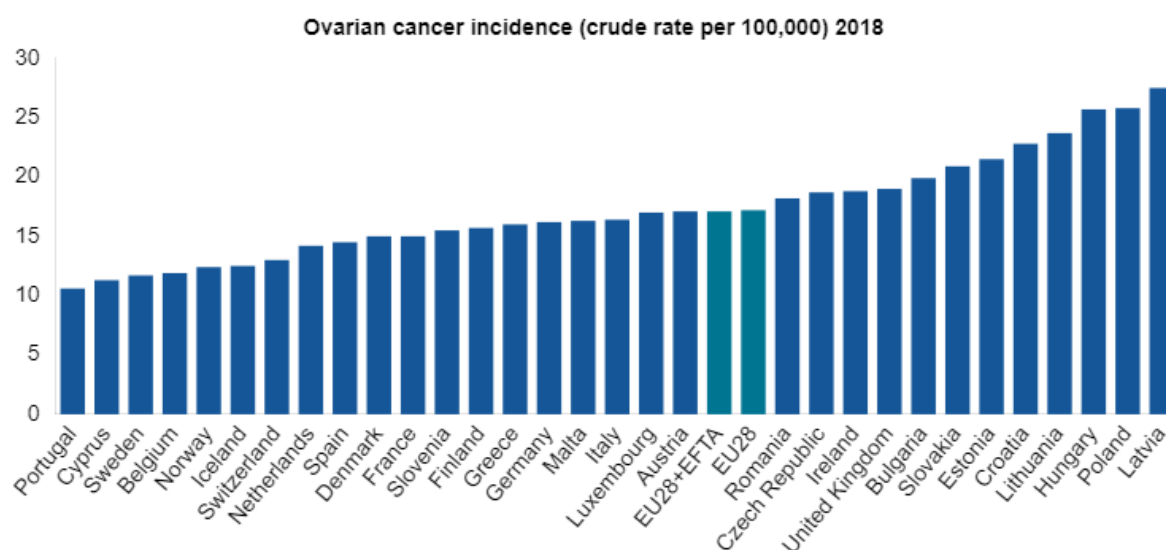


Figure 1-3 OC incidence across Europe

Taken from (85)

Ireland's aging population is contributing to an anticipated rise in OC incidence, as the disease predominantly affects women over the age of 50. As the proportion of older women increases, so too will the burden of OC. Despite its significant impact, survival rates for OC in Ireland lag behind those for other cancers (**Figure 1-4**), such as breast cancer, highlighting a need for improved diagnostic and treatment strategies(83).

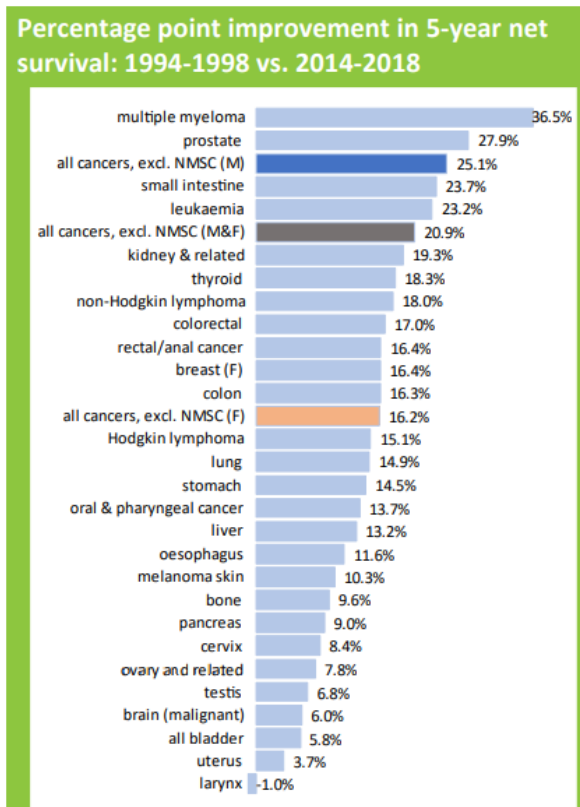


Figure 1-4 Percent Point Improvement in 5 year net survival: 1994 - 1998 vs. 2014 – 2018

Extracted from (83)

The genetic predisposition to OC, particularly related to *BRCA1* and *BRCA2* mutations, is crucial in the Irish context. Research indicates that the uptake of *BRCA1/BRCA2* predictive genetic testing in Ireland was historically low, representing a missed opportunity for early intervention and prevention (87). Efforts are underway to address these gaps. A pilot study across three tertiary cancer centers in Ireland explored the feasibility of mainstreaming *BRCA* testing for high-risk patients with breast and OC (88, 89), and the Health Service Executive (HSE) initiated a National Genetics and Genomics Strategy for Ireland in 2022 to enhance the availability and integration of genetic services, including *BRCA* testing, into the national healthcare system (90). However, implementing this strategy effectively, especially in reaching all at-risk populations, remains a challenge.

For example, fragmentation in Ireland's healthcare records poses significant challenges in managing OC. Different hospitals and clinics often use incompatible EHR systems, leading to difficulties in data sharing and continuity of care. This fragmentation is particularly problematic for OC patients, who require coordinated care across multiple specialties, including oncology, genetics, and surgery. The lack of integrated EHRs hampers research efforts by making it difficult to aggregate and analyze data across institutions. This limitation impacts patient care by causing delays in diagnosis, treatment, and follow-up.

For example, a patient's history of genetic testing may not be readily available to all healthcare providers involved in their care, potentially leading to gaps in managing hereditary cancer risks.

Ireland currently has a national cancer registry (83), a renal transplant registry (91), and is in the early stages of developing a national chronic disease registry (92). Additionally, certain medical specialties have established local disease-specific registries through their networks and efforts. Ireland also participates in European Reference Networks, contributing to international rare disease registries (93). The National Strategy for Accelerating Genetic and Genomic Medicine in Ireland recognizes the need to develop national registries for patients with genetic alterations that predispose them to cancer(94). It is recognised that these registries should be established with the involvement of Public and Patient Involvement (PPI) to ensure comprehensive and inclusive data collection and management (94).

Training and workforce development within the HSE also play crucial roles in meeting future goals for genetic services in Ireland. A 2022 survey highlighted gaps in postgraduate training in cancer genetics across multiple specialties (95), indicating a need for more comprehensive education and training programs. The HSE's 2019 review of the Clinical Genetics Medical Workforce emphasized the shortage of trained geneticists and the need for strategic workforce planning(96).

In conclusion, managing OC in Ireland is complicated by demographic shifts, genetic factors and systemic infrastructural challenges. The aging population and socioeconomic disparities necessitate targeted public health interventions to improve early detection and access to care. The genetic landscape underscores the need for enhanced genetic services and education. The lack of dedicated cancer predisposing genetic alteration registries and fragmented EHRs further impede progress in research and patient care. A multi-faceted approach, including improving genetic testing integration, developing a comprehensive OC registry, and upgrading EHR systems, is required to better address the challenges of OC and improve patient outcomes in Ireland.

1.9 Homologous Recombination Repair

1.9.1 Background

Dr Mary-Claire King was the first to show that a single gene mutation on chromosome 17q21 (then named *BRCA1*) was linked to breast and OC in many families in the 1990s, thus demonstrating a heredity component in these cancers and proving that gene mutations could predict vulnerability to these diseases (97). Her discovery revolutionized the study of cancer genetics and has proved seminal in investigating heritability of many diseases and cancer subtypes (98). The impact of germline mutations of *BRCA1* and *BRCA2* in breast and OC are now well defined (99).

1.9.2 DNA Damage Repair Pathways

Cells have evolved a complex signalling network of repair pathways known as DNA Damage Repair (DDR) to rapidly detect and repair cells against constant intrinsic and extrinsic insults (100). These vital pathways safeguard the repair of DNA to ensure the ability of cells to survive by repairing single strand breaks (SSBs) and double strand breaks (DSBs) (101), and thus maintain genomic integrity. Mechanisms for repairing DSBs include single stranded annealing (SSA), alternative NHEJ, homologous recombination (HR) and non-homologous end joining (NHEJ). Single strand break repair (SSBR), mismatch repair (MMR), nucleotide excision repair (NER) and base excision repair (BER) are mechanisms that ensure SSBs are repaired (102). HR, considered a *BRCA1/2* dependent pathway, is the preferred repair pathway for DSB for *BRCA1/2* proficient cells (103, 104). In this pathway, HR exploits the duplicate genetic data located on the homologous sister chromatid. During the S-phase of DNA replication HR uses the sister chromatid as a reference to restore nucleotides of damaged DNA in the instance of replication fork stalling (105, 106) as depicted in **Figure 1-5** extract from McNevin et al (100).

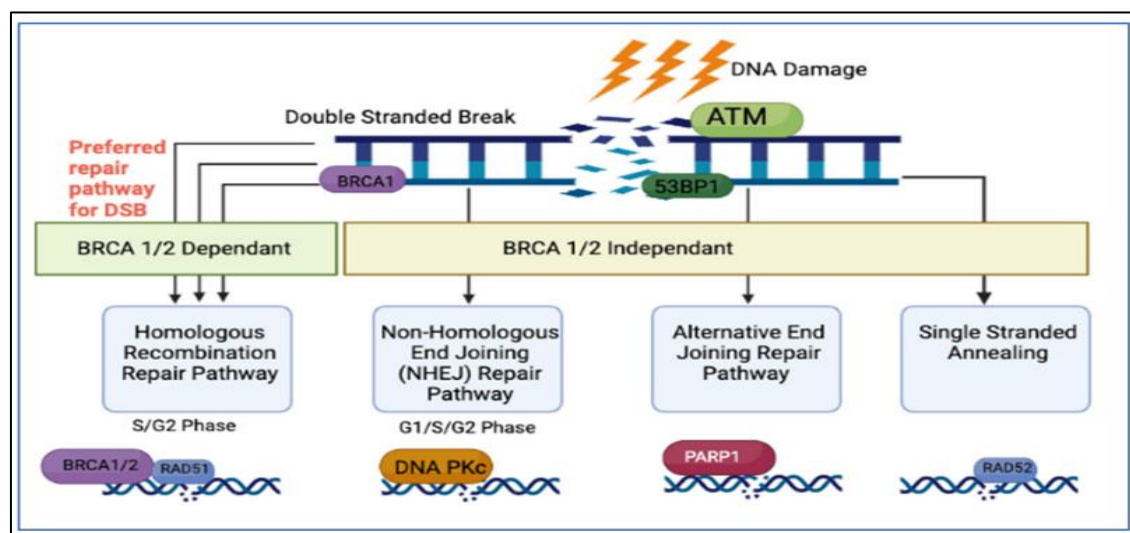


Figure 1-5 Repair Pathways for DSBs

Taken from source (71)

BRCA1 promotes end resection with *BRCA2* loading *RAD51*, which in turn facilitates error free repair by catalysing the homology search on the sister chromatid (107). The presence of sister chromatids is limited to S and G2-phase, therefore cells are unable to utilise HR throughout the whole cell cycle. The absence of a complete repair template in G1-phase means cells mainly depend on the *BRCA1/2* independent NHEJ for the repair of DSBs (103). *BRCA1/2* deficient cells, which regulate S and G2-phase also get shunted towards *BRCA1/2* independent pathways (104). Pathway selection is regulated by the presence or absence of phosphorylation of 53BP1 by the ATM serine/threonine kinase that initially recognizes DSB. The decision as to whether to initiate end-resection (i.e., HR pathway) or block end-resection (i.e., end-protection resulting in NHEJ), is determined by either the presence of 53BP1 or by *BRCA1* and *CtIP* (103). Genomic instability demonstrated in malignant cells drives the accumulation of mutations. This in turn prompts the development of tumour heterogeneity (108). Mutations in DDR genes can compromise a cells integrity, the significance of which is emphasized by numerous human syndromes (i.e., Lynch syndrome and hereditary nonpolyposis colorectal cancer).

1.9.3 HRD and OC

Understanding the genetic drivers of OC has led to advances in therapeutic options and survival rates. In summary, HR proficiency appears to be lost in 50% of HGSOC, with approximately 30% loss due to germline (14%), somatic (6%), or epigenetic (10%) loss of *BRCA1* or *BRCA2* function (epigenetic changes are reported for *BRCA1* only) (3).

1.9.4 BRCA contributors to HRD in OC

It is estimated that more than 50% of HGSCOs contain defects in HR repair at the time of diagnosis, the most prominent cause being *BRCA* mutations, the distribution of which is outlined below in **Table 1-6**. Epigenetic silencing of *BRCA1* via promoter hyper methylation results in HRD in a further 5–20% of HGSCOs, a process, which is mutually exclusive with *BRCA1/2* PV. In a recent study of nearly 10,000 *BRCA* mutation carriers, the cumulative risks of developing OC prior to age 80 years were estimated to be 44% (95% confidence interval (CI) 36–53%) for *BRCA1* and 17% (95% CI 11–25%) for *BRCA2* (27). This compares to 1.3% in the general population (109), highlighting the importance of these genes in the development of OC.

	Germline	Somatic	References
<i>BRCA1</i> Mutation	8-12%	5-7%	(49, 110, 111).
<i>BRCA2</i> Mutation	3%	3%	

Table 1-6 Frequency of BRCA Mutations in newly diagnosed HGSOC

1.9.5 Other HR-associated mutations in OC

As stated above, it has been estimated that approximately half of HGSOCs contain defects in HR DNA repair of which germline or somatic *BRCA1/2* mutations account for approximately 20% (49, 110, 111). The Cancer Genome Atlas (TCGA) study attributed the remaining 30% to other alterations, including mutations in Fanconi anaemia genes (*FANCC*, *FANCD2*, *FANCA*, *FANCE*, *FANCI*, *FANCL*, and *FANCM*), other genes associated with DNA damage repair and genome stability (*ATM*, *ATR*, *CHEK2*, *NBN*, *PALB2*, *RAD50*, and *RAD51*), *BRCA1* promoter hypermethylation, *EMSY* amplification, and loss of *PTEN* (5). For instance, women with germline mutations in the genes *BRIP1*, *RAD1C* and *RAD1D* have estimated lifetime risks of developing OC of 5.8%, 5.2% and 12%, respectively (112). The common link between all these genes is their role in the HR mediated pathway of DNA repair, which is known to play a prominent role in the pathophysiology of HGSOC. Approximately 30% of HGSOC may have other alterations that do not cause HRD but still render tumours sensitive to PARPis (e.g. *NER* mutations and *MMR* mutations); these are under study (3). Fifteen percent of HGSOCs have cyclin E1 (*CCNE1*) amplification and are not sensitive to PARPis (49). A breakdown of genetic drivers in HGSOC taken from Konstantinopoulos et al (49) is shown in **Figure 1-6**.

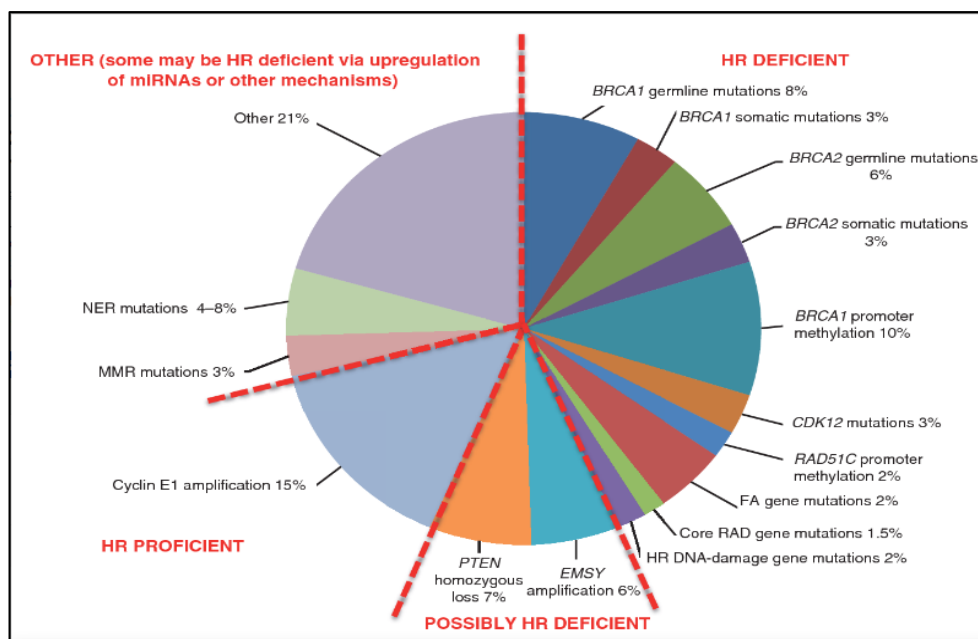


Figure 1-6 Genetic breakdown in HGSOC

Taken from (6)

1.10 miRNA and OC

1.10.1 miRNA dysregulation in OC

miRNAs are a class of small non-coding RNAs having an average of 22 nucleotides in length. Discovered over 30 years ago in 1993 (113), miRNA can be found in tissue and body fluids and regulate the expression of around 30% of human genes (114). They function by controlling transcriptional and translational processes and thus they influence and control both physiological and pathological processes, such as development, cell differentiation, proliferation, apoptosis and tumour growth (115).

miRNAs have been shown to be involved in several biological actions such as apoptosis, response to oxidative stress conditions, proliferation, migration, differentiation, haematopoiesis, drug resistance and cell cycle regulation (116). Perhaps the most relevant to cancer development and growth, miRNA have been shown to be involved in regulation of the epigenetics modifiers, including DNA methyltransferases and targeting of epigenetic modifications (DNA methylation). As miRNAs can be altered due to pathological processes (117), observed dysregulation of miRNA can be associated with cancer initiation, progression, and metastasis (118). While miRNAs are widely recognized for their role in negatively regulating target mRNA (119), they have also been linked to the promotion of gene expression through various mechanisms. These include miRNA-host gene co-expression, inhibiting upstream suppressor, co-regulation by shared transcription factors, and targeting gene enhancer (120).

It is worth stating early that the sensitivity and specificity of miRNA in identifying pathological states such as cancer from normal physiology can be inconsistent (121). Several miRNAs associated with OC have also been shown to be either up- or down-regulated in different studies. For example, miR-124-3p, miR-148a-3p, miR-203a, and miR-375 were detected demonstrating both downregulation and upregulation in OC specimens compared to normal tissue (122). Even miRNA that have been shown to be associated with a particular disease state, shows conflicting data in the literature. For example Márton *et al.* demonstrated that the sensitivity and specificity of miR-429 to diagnose OC were 0.857 and 0.683 respectively (123), while Meng *et al.* found that the sensitivity and specificity were 0.594 and 0.955, respectively (124, 125). The reason for such inconsistencies are uncertain; however, it may be related to sample size and/or heterogeneity within tumor samples or between different EOC subtypes (122).

To add complexity, it has been reported that a single miRNA may bind to over 200 target genes, and one gene can be targeted by many miRNAs (126). This multifaceted relationship between miRNA-mRNA, makes it challenging to identify target transcripts and pathways that are regulated by specific miRNAs (120). Moreover, there is no consensus on the best approach for miRNA detection, data analysis, and standardization of methodologies (127). This absence of standardization is reflected across numerous miRNA and OC studies. Some investigators compared miRNA expression in ovarian serous adenocarcinoma

tissues to normal ovarian tissue (128) while others investigated the differences between recurrent vs. primary OC tissue (129). Studies have been conducted looking at the correlation of miRNA and mRNA expression profiles and OS in OC (120), while others have focused on investigating miRNAs as a marker of response of tumour cells to chemotherapeutic agents (6). Even within each of these studies, OC can be represented by a heterogeneous group of histopathologies (HGSOC, MOC, CCOC, LGSOC) (120), which are clinically distinct. Despite many examples of miRNAs as markers for prognosis in OC (118, 122), to date, this has largely remained in the academic field, and has yet to be translated into clinical utility.

Changes in the levels of specific miRNAs—either upregulation or downregulation—in tumors and bodily fluids have been associated with alterations in cell migration, invasion, and metastasis. The question is whether these miRNAs can be used as potential biomarkers in clinical settings. miRNAs are promising candidates because they can be easily detected in tumor tissue and various body fluids, including plasma, serum, urine, saliva, ascites, and pleural fluid (121). Despite recent advancements in technologies allowing for the use of miRNA microarrays and high-throughput deep sequencing techniques, have aided in advancing our knowledge of miRNA, (130), identifying miRNA biomarkers with for specific cancer states remains a challenge.

1.10.2 miRNA associated with tumour suppression in OC

Several miRNAs act as tumor suppressors by downregulating oncogenes or genes involved in promoting tumor progression. A review paper by Nguyen et al., listed over 80 miRNAs related to over 90 associated genes, that have been reported to be down-regulated in OC (122). Prominent illustrations include miR-34a and let-7 family. Downregulation of these miRNAs in OC contributes to uncontrolled cell proliferation, invasion, and metastasis.

1.10.3 miR-34a as a tumour suppressor in OC

miR-34a is a well-studied tumor suppressor that is frequently downregulated in OC (131, 132). It regulates various processes involved in tumor suppression, including cell cycle arrest, apoptosis, and inhibition of metastasis (133). In a study by Welponer et al. (132), significantly lower levels of the miR-34 family were demonstrated in OC compared to control-tissues, and suggested that downregulation of miR-34 family is a crucial part in OC development.

Most interestingly, miR-34 family is transcriptionally induced by *TP53* (**Figure 1-7**). *TP53* is driver mutation in the pathogenesis of HGSOC and is almost ubiquitously mutated in HGSOCa tumours (134). It has been suggested that mutations in *TP53* gene may favour carcinogenesis and tumor proliferation by reduced levels of intracellular miR-34 family members (135).

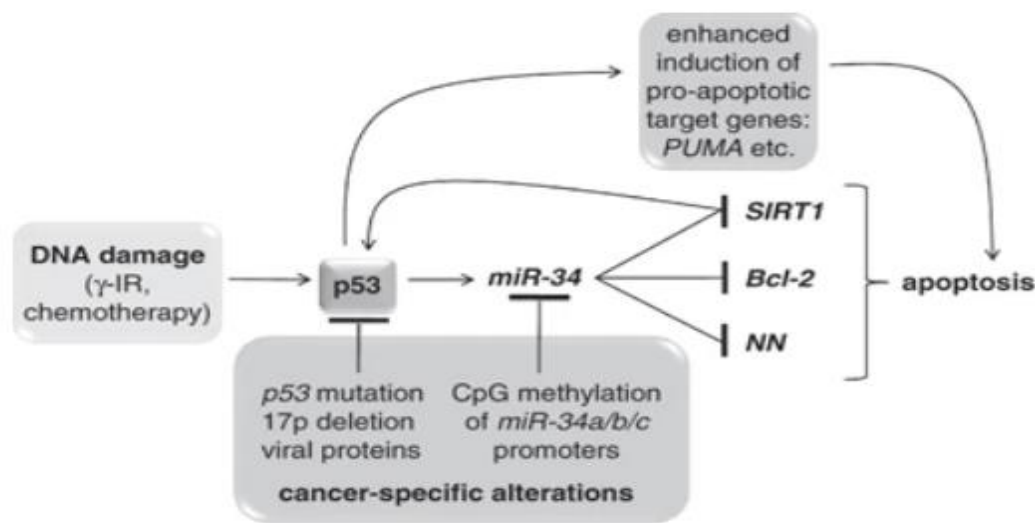


Figure 1-7 The p53/miR-34 pathway regulates apoptosis and is altered in cancer

Taken from Hermeking et al (133)

The human lethal-7 (let-7) family, consisting of 13 members, has an important role in the regulation of cell proliferation and carcinogenesis (136). Let-7d has garnered attention in OC research due to its potential diagnostic and therapeutic significance, with studies revealing dysregulation of let-7 family members, including let-7d, in OC patients (136), suggesting their utility as diagnostic markers. Let-7d dysregulation is associated with various aspects of OC pathogenesis, including metastasis, response to chemotherapy, and cancer stem cell phenotype regulation. For instance, let-7d-3p is associated with apoptosis and positive response to carboplatin/paclitaxel chemotherapy in patients with OC (137). Further, down regulating let-7b has been shown to promote cancer progression in OC (138). Let-7e has also been reported to sensitise epithelial OC to cisplatin through repression of DNA double strand break repair (139).

Mechanistic studies have identified targets of let-7d, such as HMGA2, KRAS, c-Myc, and HMGA1, implicating its involvement in key signalling pathways driving tumor progression and drug response. The regulation of Let-7 expression involves various transcriptional and post-transcriptional mechanisms. These include transcriptional control by OCT4, MYC, and mutant p53, as well as posttranscriptional regulation by the RNA binding protein LIN28 and the RNA editing enzyme ADAR (136). It has been reported that the let-7 family regulates RAS (140), in turn regulating K-RAS mRNA and therefore the cell proliferation is reduced upon let-7d upregulation. Abnormal expression of let-7 family members in patients with OC compared with that in healthy controls was identified in several studies and so the differentially expressed miRNA may have potential as a diagnostic marker (136, 137, 139).

1.10.4 miRNA associated with tumour promotion in OC

Certain miRNAs have been identified as playing a crucial role in OC progression. A systematic review by Nguyen et al. (122) outlined 38 miRNAs associated with 'pro metastatic' tumours. miR-21 (141), miR-182 (142, 143), miR-23a (144), miR-301b-3p (145), and miR-130a (146) are a few examples of commonly identified miRNAs shown to be upregulated in OC tissues and cell lines. These miRNAs contribute to tumor growth, metastasis, and chemoresistance by targeting tumor suppressor genes and dysregulating various signaling pathways. For instance, miR-21 targets PTEN, miR-155 targets FOXO3a and TP53INP1(141), and miR-182 targets MTSS1, FOXO3, and BRCA1, promoting oncogenic signaling and inhibiting apoptosis. Additionally, miR-222 targets p27, TIMP3, and PTEN, while miR-23a targets APAF1 and PTEN, leading to dysregulated apoptosis and PI3K/AKT signaling. Moreover, miR-301b-3p targets PTEN, CDKN1A, and NFAT5, while miR-130a targets PTEN, GAX, and RUNX3, activating the PI3K/AKT and MAPK pathways and promoting tumor cell proliferation, migration, and invasion.

1.10.5 miR-200 Family as a tumour promoter in OC

miR-200 family members, including miR-200a, miR-200b, and miR-200c, have emerged as significant players due to their altered expression patterns observed in cancerous tissues compared to healthy counterparts (147). These miRNAs are organized into two distinct groups on the chromosome, and each group contains multiple copies of hairpin precursors from which the mature miRNAs are derived. Each precursor can generate both a mature -5p and -3p miRNA, contributing to the diversity and regulatory potential of this family (148). Studies, such as that by Braicu et al., have highlighted increased expression levels of various miR-200 family members, such as miR-200c, miR-141, and miR-200b, in formalin-fixed cancerous tissues (147). Notably, elevated expression of miR-200a, miR-200b, and miR-200c has been consistently linked to poor prognosis in OC patients as evidenced by numerous experimental studies (149-152) and through multi omic in silico work (153). However, it has also been reported that members of the miR-200 family have been shown to have both pro- and anti-metastatic effects (122). For example, miR-200a has been reported both upregulated (154-156) and downregulated (157-159) in OC compared to normal tissue, which echoes the complexity of miRNA studies. Further, the miR-200 family has been associated with regulation of EMT.

1.10.6 miRNA associated with EMT in OC

Epithelial-Mesenchymal Transition (EMT) is a crucial process in cancer metastasis, where epithelial cells acquire mesenchymal characteristics, allowing them to invade surrounding tissues and migrate to distant sites (160). These mesenchymal characteristics include the downregulation of epithelial cadherin (E-cadherin, CDH1) and the upregulation of mesenchymal neural cadherin (N-cadherin, CDH2) (161). E-cadherin, a transmembrane glycoprotein, forms complexes with β -catenin at adherent junctions. The loss of E-cadherin function leads to the disruption of adherent junctions, facilitating cellular migration, invasion,

and ultimately metastasis (122). An association between numerous miRNAs and OC metastasis, through targeting various genes and pathways to induce epithelial-mesenchymal transition (EMT), has been reported (122), see **Figure 1-8**.

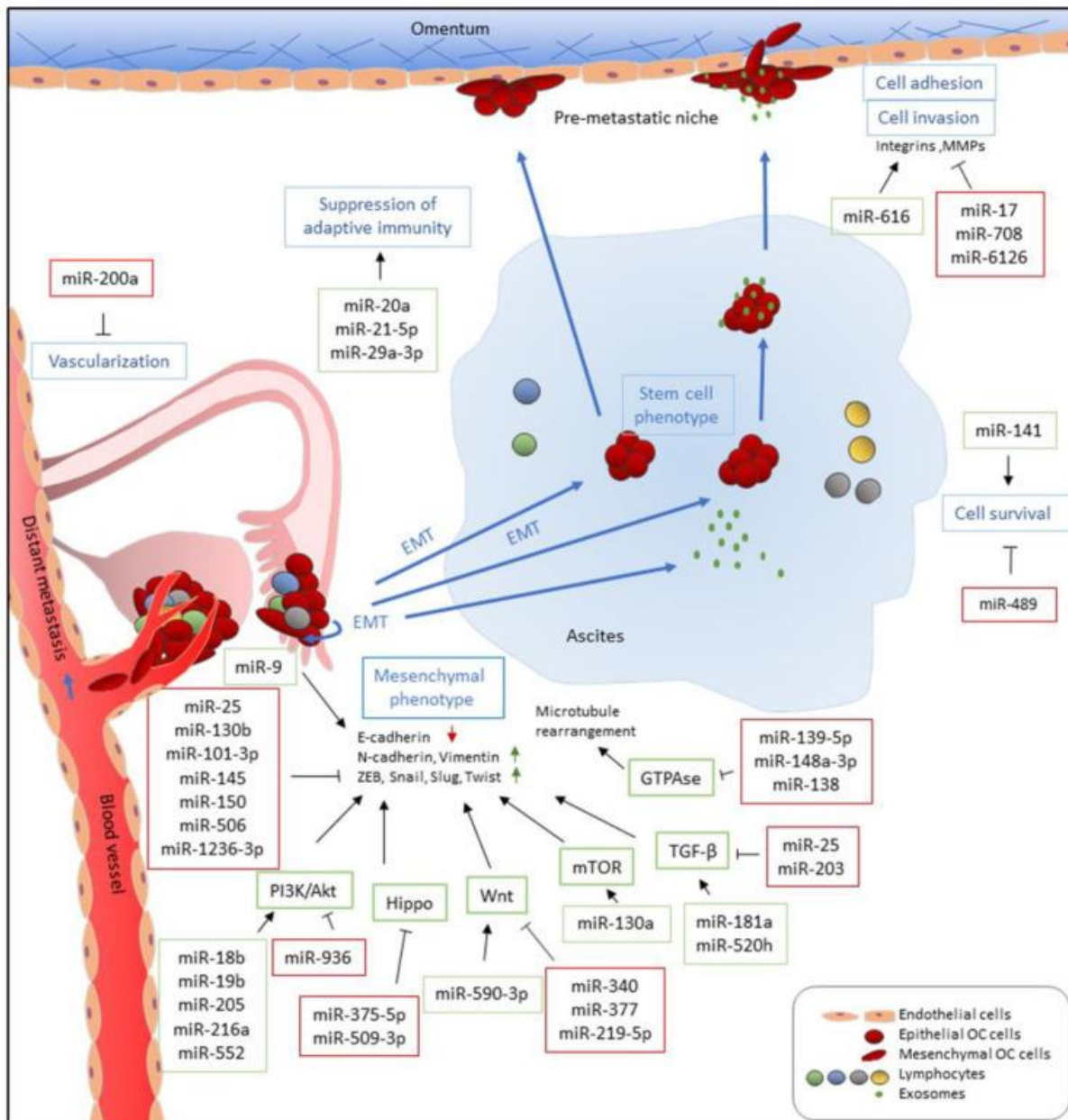


Figure 1-8 Functions of miRNAs in EOC metastasis

1.10.7 miR-200 Family and their role in EMT in OC

The miR-200 family plays a crucial role in regulating the epithelial-to-mesenchymal transition (EMT), a process fundamental to cancer progression and metastasis (148). Through intricate molecular mechanisms, miR-200 members counteract EMT by targeting key transcription factors such as ZEB1 and ZEB2, thereby promoting the expression of epithelial markers like E-cadherin (162, 163). Additionally, miR-200 directly

influences the expression of various cell adhesion molecules, signaling proteins, and components of the extracellular matrix, further modulating the cellular phenotype. Beyond ZEB proteins, miR-200 targets several other drivers of the EMT program, including TGFBR1, SMAD2, ETS1, Notch signaling components, PBX3, c-Myb, and co-factors in repressive complexes (162). Moreover, miR-200 impacts critical regulators of gene expression and cellular processes, such as QKI-5, SIRT1, MYC, and the long non-coding RNA HOTAIR. Functionally, miR-200's influence extends to NF- κ B activation, JUN expression, mitochondrial transcription, and cancer cell stemness. Furthermore, miR-200 can exert effects beyond cell boundaries through its presence in extracellular vesicles, highlighting its potential as a therapeutic agent in cancer treatment. This multifaceted regulatory network underscores the significance of miR-200 in tumor suppression and its potential as a therapeutic target in OC (114, 164) .

1.10.8 miR-9 and its role in EMT in OC

There have been a number of studies which have implicated miR-9 in the promotion of malignancy (165), specifically in OC. Several studies, including those by Zhou et al. (166) and Sui et al. (167), have linked miR-9 to metastases in OC. Zhou et al. found miR-9 to be upregulated in metastatic sites compared to primary sites of OC, showing a negative correlation with E-cadherin mRNA and a positive correlation with vimentin mRNA expression. Additionally, Sui et al. demonstrated that miR-9 promotes the EMT of OC cells by targeting E-cadherin (CDH1), indicating its involvement in facilitating cancer cell migration and invasion, given E-cadherin's established role in EMT (168).

These findings underscore the potential prognostic relevance of miRNA dysregulation in OC, suggesting its involvement in disease progression and highlighting its potential as a biomarker for assessing patient outcomes and guiding treatment strategies. Evidently, the role miRNA play in these processes are multifaceted and complex. The challenge therefore is to uncover crucial insights into the molecular mechanisms driving OC pathogenesis and potentially paving the way for novel avenues in prognosis, diagnosis, and treatment through the identification of a critical biomarker.

1.11 circRNA and OC

While Homologous Recombination Deficiency (HRD) highlights critical vulnerabilities in cancer cells, recent studies suggest that circular RNA (circRNA) may play a significant role in regulating these vulnerabilities (169) and influence HRD-related pathways, potentially offering new insights for therapeutic intervention(170). circRNA, a type of non-coding RNA, were previously thought to be a non-functional by-product of pre-mRNA missplicing and largely disregarded (169, 171). More recently, circRNAs have been proven to play important roles in biological settings, to manipulate the functions of molecules, including non-coding RNAs, mRNAs, DNAs and proteins to regulate cell activities in physiology and pathology (169, 172). Accumulating evidence also indicates that circRNAs play critical roles in tumorigenesis, tumour development (173-176) and sensitivity to anti-cancer treatments (170, 177-179). In addition, unlike linear RNAs, the circular structure is exonuclease resistant, a property accounting for high stability in the circulation and conferring huge potential as a circulating biomarker (178-180). circRNAs have been identified as having important prognostic, diagnostic and therapeutic potential in OC such as such as serving as biomarkers for early detection, predicting disease progression and response to treatment, and providing novel targets for targeted therapies and personalized treatment strategies. (58, 181-183). However, an assessment of circRNAs in OC focusing on *BRCA* status is novel. In this regard, previous studies have focused on cell lines, which may, or may not be truly representative of HGSOC (182, 184).

1.11.1 Background

Approximately 1–2% of the human genome is transcribed into RNA transcripts, which are then translated into protein (185). Messenger RNA (mRNA), which accounts for 3–7% of RNA in mammalian cells(186), is involved in protein synthesis. However, the majority of RNA subtypes are non-coding. These include ribosomal RNA (rRNA), transfer RNA (tRNA) (10–15%), long non-coding RNA (0.06–0.2%), microRNA (miRNA) (0.003–0.02%) and circular RNA (circRNA) (0.002–0.03%)(185). Viroids, small, circular, single-stranded RNA molecules consisting of about 246–401 nucleotides were the first circRNA molecules to be discovered by Sanger et al. in the late 1970's, and were initially degraded as cellular 'junk' (187). Later, in 1991, when circRNAs were observed in the cytoplasmic fractions of eukaryotic cell lines by electron microscopy, they were considered a non-functional by-product of pre mRNA missplicing and discounted (169, 171, 188). Being covalently closed, without polyadenylation at their 3' ends, most circRNA were undetectable in transcriptomic based polyadenylated RNA profiling (189).

The discovery that circular transcripts of cerebellar degeneration-related protein 1 antisense RNA (CDR1as, also known as ciRS-7) can serve as miRNA sponges for miR-7 (190, 191) was a major turning point (178). Further advances in high-throughput sequencing technologies (RNA-Seq) and bioinformatics methods have provided the framework to interrogate circRNA (173, 188). Millions of circRNAs have now been identified in multiple cell lines and species (192, 193) and have been demonstrated as an abundant and conserved class

of RNAs (188).

circRNAs have been identified to manipulate the functions of diverse molecules, including non-coding RNAs, mRNAs, DNAs and proteins to regulate cell activities in physiology and pathology (169, 172). In addition, unlike linear RNAs, the circular structure is exonuclease resistant, a property accounting for high stability in the circulation and conferring huge potential as a circulating biomarker (178-180).

1.11.2 Biogenesis of circRNAs

Various models of circRNA formation have been proposed; lariat-driven circularization and intron-pairing-driven circularization(58). The distinction between these two models, focuses on what step, canonical splicing or back-splicing, happens first (183, 189). Emerging evidence has revealed circRNAs are generated by the back-splicing process, in which a downstream donor splice site is covalently bonded to an upstream splice acceptor site producing a circular transcript (183, 189). The joining of these two ends forms a closed continuous loop structure without 5' or 3' tails, creating its circularity. This contrasts to linear messenger RNA (mRNA), which is formed through canonical eukaryotic pre-mRNA splicing.

circRNAs can be roughly categorised into three types according to their biogenesis pattern (173); exonic circRNAs (ecircRNAs) contain exons, intronic circRNAs (icircRNAs) contain introns, and exon-intron circRNAs (eicircRNAs) contain both exons and introns (194). Among circRNAs, ecircRNAs are the most common, representing more than 80% of identified circRNAs (173). These are formed by exon skipping during pre-messenger RNA (pre-mRNA) transcription to produce a lariat structure containing exons, which is then spliced internally to release introns and form EciRNAs composed of exons (192). This lariat-driven circularization is the first model of circRNA formation. Alternatively, the tail end of the downstream 3' splicing donor site in the exon binds to the upstream 5' splicing receptor site, resulting in base pairing of the donor and receptor sites, which mediates exon circularization to form circRNAs. This is the intron-pairing-driven circularization.

1.11.3 Characteristics of circRNA

circRNAs have distinctive characteristics. Firstly circRNAs are found abundantly in eukaryotic cells (194) exceeding the presence of related linear mRNAs in human cells (173), with greater than one million circRNAs identified in human tissue (178, 193). As described above, their closed ring structure created by the bond between the 3' and 5' ends, means they do not have a polyadenylated tail open to degradation by exonucleases and therefore much more stable than linear RNA (178, 195). *Jeck et al.* reported that the average half-life of circRNAs in most species is more than 48 h, while the half-life of mRNAs on average is about 10 h (196). Further, circRNAs tend to be located in the cytoplasm rather than the nucleus (191). The majority of circRNAs demonstrate conservatism, meaning that they preserve sequences between diverse species. For example, many circRNAs have been identified in both humans and mice (193, 197). circRNAs

display tissue-specific and dynamic developmental stage-expression patterns and play a regulatory role at the level of transcription or post transcription. Their important role in biology as miRNA sponges (190, 198) gene transcription and expression regulators (173, 196), RNA-binding proteins (RBP) sponges (199), and ability to encode peptides (200, 201) suggest that circRNAs are very important warranting further investigation.

1.11.4 Clinical Application of circRNA in OC

Recent studies have confirmed the significant biological functions of circRNAs, especially in human cancers (169, 170, 185). There have been publications in the past years that have summarised the role of circRNA in OC (58, 183). Some have been identified as having oncogenic roles, whereas others play tumour suppressive roles (170, 202). Therefore, selected circRNAs have the potential to be used in OC as a predictive biomarker, a diagnostic biomarker, as a marker of drug resistance or as a potential drug target.

1.11.5 circRNAs as Potential Biomarkers in EOC

A number of circRNA's have been identified as a potential predictive biomarker and / or as a diagnostic biomarker from cell line studies (**Table 1-7**). For instance, circHIPK3 and circLARP4 have been identified as prognostic biomarkers, with their expression levels correlating with disease progression and patient outcomes. Additionally, circEXOC6B and circN4BP2L2 have emerged as promising diagnostic markers due to their dysregulation in OC tissues. Collectively, these findings underscore the utility of circRNAs in improving our understanding of OC and enhancing clinical management strategies.

1.11.6 circRNAs and Drug Resistance in OC

A number of circRNAs have been identified in drug resistant OC, particularly cisplatin resistance (**Table 1-8**). While no circRNA signatures relating to PARPi resistance have been identified as of yet, a recent study has outlined key regulatory networks for PARPi treated cell lines and their involved pathways in the PARPi resistant OC cells (203).

CircRNA	Study Finding	Study Methodology; Translational Arm	Cell Line used	Biomarker Potential	Reference
circHIPK3	Silencing of circHIPK3 promoted proliferation, migration, invasion and inhibited apoptosis of OC cells. The circHIPK3-miRNA-mRNA axis was predicted as a possible mechanism using bioinformatic approaches.	21 Normal Ovarian Tissue samples matched to 21 High Grade EOC	A2780, SKOV3	Drug target	Teng et al., 2019 (204);
	circHIPK3 was upregulated in EOC tissues and cells compared to adjacent normal tissue and ovarian epithelium cell line, respectively. Higher circHIPK3 expression was associated with lymph node invasion, FIGO stage, and worse DFS and OS. Moreover, analysis showed that higher circHIPK3 was an independent predictor of DFS and OS in EOC patients.	69 EOC tissues and adjacent noncancerous tissues were obtained from primary EOC	A2780 , SKOV3, HO8910; CAOV3	Prognostic biomarker	Liu et al., 2018 (205)
circEXOC6B	circBNC2, circEXOC6B, circFAM13B, circN4BP2L2, circRHOBTB3 and circCELSR1 were significantly dysregulated in EOC specimens. It was also reported that circEXOC6B and circN4BP2L2 may serve as diagnostic biomarkers.	54 EOC specimens and 54 normal ovarian tissues (controls)	NA	Diagnostic and prognostic biomarker	Ning et al., 2018 (206) <i>Continued...</i>
circN4BP2L2					

CircRNA	Study Finding	Study Methodology; Translational Arm	Cell Line used	Biomarker Potential	Reference
circRNA 1656	circRNA1656 was down-regulated in both HGSOC tissue and OC cell lines, and was significantly associated with HGSOC FIGO stage, and is expected to become a novel tumour marker for HGSOC.	60 cases of OC cases were HGSOC; 60 cases in the control group	A2780 , SKOV3, HO8910, OVCAR-3	Diagnostic biomarker	Gao et al., 2019 (207)
hsa_circ_0003972 hsa_circ_0007288	The expression of hsa_circ_0003972 and hsa_circ_0007288 (circCOMBO) was down-regulated in cell lines, and in plasma and tissues of OC patients. Among them, high expression levels of hsa_circ_0007288 in plasma and OC tissues were related to lower lymph node metastatic potential in OC.	41 OC tissues and 15 adjacent tissues were obtained from OC patients	A2780 , SKOV3	Diagnostic and prognostic biomarker	Ge et al., 2022 (208)
Circ_0078607	circ_0078607 suppressed OC progression by sponging oncogenic miR-518a-5p to induce Fas expression	9 pairs of OC and adjacent non-cancerous tissue samples	A2780 , SKOV3	Drug Target	Zhang N. et al., 2020 (209)
circLARP4	circLARP4 expression was lower in OC tissues. Lower circLARP4 expression was associated with poor prognosis of OC patients. This also correlated significantly with FIGO staging and lymph node metastases.	78 paired OC tissue and adjacent normal tissue samples	No cell lines were used	Diagnostic and prognostic biomarker	Zou et al., 2018 (210) <i>Continued...</i>

CircRNA	Study Finding	Study Methodology; Translational Arm	Cell Line used	Biomarker Potential	Reference
VPS13C--hsa-circ-001567	Significantly promoted apoptosis and suppressed proliferation of SKOV3 and OV-1063 cells.		SKOV3 and OV-1063 cells		
circFoxp1	Upregulation of circFoxp1 was an independent predictor of survival outcome.	112 patients with EOC and 82 healthy people	COC1, OVCAR3, SKOV3, SKOV3/DDP	Prognostic biomarker	Luo and Gui, 2020 (211)
circITGB6	circITGB6, robustly elevated in tumour tissues and serums from patients with platinum resistant OC and was correlated with poor prognosis.	10 OC tissues (5 chemo-resistant tissues vs. 5 chemo-sensitive tissues)	OVCAR3, SKOV3, TOV21G, TOV112D and CAOV3	Prognostic biomarker	Li et al., 2022 (212)
hsa-circ-0061140	Identified interactions among hsa_circ_0061140, miR-370, and FOXM1 that potentially contributed to OC development.	Nil	KOV3, A2780, OV2008, IGROV1, and ES-2	Prognostic biomarker	Chen Q. et al. (2018) (213)

Table 1-7 circRNA as Biomarkers in OC

circRNA	Study Finding	Study Methodology; Translational Arm	Cancer Cell Line used	Drug Resistance	Reference
circLPAR3	Study reported circRNA significantly upregulated in cisplatin (DDP) -resistant OC tissues. circ-LPAR3 may be involved in DDP resistance in facilitating OC via the miR-634/PDK1 axis	66 OC patients (Cisplatin sensitive patients (n = 33) and resistant patients (n = 33))	A2780 , SKOV3 and A2780r/DDP SKOV3r/DDP	Cisplatin	Liu et al., 2022 (214)
circITGB6	circITGB6 overexpression promoted an M2 macrophage-dependent cisplatin resistance in both vivo and vitro.	10 OC tissues (5 chemo-resistant tissues vs. 5 chemo-sensitive tissues)	OVCAR3, SKOV3, TOV21G, TOV112D and CAOV3	Cisplatin	Li et al., 2022 (212)
circFoxp1	circFoxp1 confers DDP resistance in EOC cells. circFoxp1 can be used as a biomarker and potential therapeutic target for EOC.	112 patients with EOC and 82 healthy people	COC1, OVCAR3, SKOV3, SKOV3/DDP	Cisplatin	Luo and Gui, 2020 (211)
circPLEKHM3	circPLEKHM3 functions as a tumour suppressor in OC cells by targeting the miR-9/ <i>BRCA1</i> / <i>DNAJB6</i> / <i>KLF4</i> / <i>AKT1</i> axis and may be used as a prognostic indicator and therapeutic target in OC patients.	5 tumour tissues from OC patients and 5 normal ovarian tissues from patients with benign gynaecological diseases were collected	A2780, OV90, MDAH2274	Taxol resistance when combined with AKT inhibitor (MK- 2206)	Zhang L. et al., 2019 (215)
hsa_circ_0063809	circCELSR1 is frequently activated in Taxol resistant OC tissues and cell lines and is associated with aggressive OC phenotypes.	36 ovarian carcinoma specimens were collected from OC patients	SKOV3 and HeyA-8	Taxol resistance	Zhang N. et al., 2020 (216)

Table 1-8 circRNA and Drug Resistance

1.11.7 CircRNAs relating to BRCA

There is little in the literature at this time characterising circRNA as it relates to *BRCA* status in OC.

Only a few studies have described the profile of circRNA in BRCA by microarray analysis, however this specifically looked at a breast cancer cohort (217). In a separate study, circPLEKHM3 was identified as having an oncogenic effect in OC cells by targeting the miR-9/ *BRCA1* / DNAJB6 / KLF4/AKT1 axis (215). There have also been a number of more tenuous links made. For instance, hsa_circ_0000199 was reported to activate the DNA repair molecule histone family member X (H2AX), leading to resistance to cisplatin in breast and gastric cancer models (218). Given that *BRCA* is embedded in the regulation of the DNA repair pathways, this may be relevant, however, the specific nuances around the relationship between *BRCA* and specific circRNAs associated with resistance need to be further investigated.

1.11.8 circRNA – miRNA Networks

1.11.8.1 Background

miRNA are a class of small non-coding RNAs having an average of 22 nucleotides in length.

Discovered over 30 years ago in 1993 (113), miRNA can be found in tissue and body fluids and regulate the expression of around 30% of human genes (114). They function by controlling transcriptional and translational processes and thus they influence and control both physiological and pathological processes, such as development, cell differentiation, proliferation, apoptosis and tumour growth (115). circRNAs, as discussed previously, are the covalently closed RNAs synthesized when the pre-mRNA transcripts undergo a back-splicing event (219). Progressions in high-throughput RNA sequencing and bioinformatics tools, have allowed for exploration of the circRNA regulatory pathways, one of which is miRNA-mediated regulation(220). The interaction between miRNAs and circRNAs involves multifaceted and complex regulatory networks. Below is a table highlighting differing general characteristics. It is important to note that there can be exceptions, and the roles of both miRNAs and circRNAs are areas of ongoing research with expanding knowledge. Variations in miRNA expression appear to influence cancer development, and there is much evidence to support that miRNAs play a role in the occurrence, migration, and invasion of tumours (221). circRNA can act as miRNA sponges, and indirectly regulate the expression of miRNA target genes. They do this by containing binding sites for specific miRNAs, which function as decoys to sequester miRNAs away from their target messenger RNAs (mRNAs)(222). An example of this which has been extensively studied is a circRNA, known as cerebellar degeneration-related 1 antisense transcript (CDR1as), which has over 70 binding sites of miR-7, and exerts its function primarily through sponging of miR-7 (223). Besides miR-7, CDR1as can also act as a sponge for several other miRNAs (Table 1-9). These miRNAs have been associated with different target genes, and have been reported as possibly tumour promoting or tumour inhibitory (223). Therefore, while CDR1as regulatory role in tumour development is accepted, its relationship is complex and requires further investigation. This example

mirrors much of the literature to date. For instance, several studies have identified the circRNA-miRNA-mRNA axis that activates pathways associated with various cancers (224), however each process is complex and unique.

CDR1as sponge miR	Cancer	Target gene	Tumor Progression
miR-7	Breast Cancer	CCNE1	promoting
	Osteosarcoma	EGFR, CCNE1, PI3KCD,RAF1	promoting
	Laryngeal squamous cell carcinoma	proliferation indices ki-67, CCNE1, PIK3CD	promoting
	Non Small Cell Lung Cancer	proliferation indices Ki-67, EGFR, CCNE1, PIK3CD	promoting
	Colorectal Cancer	EGFR, IGF-1R	promoting
	Pancreatic Ductal Adenocarcinoma	EGFR, STAT3	promoting
	Hepatocellular carcinoma	EGFR	promoting
		CCNE1, PIK3CD	promoting
		PIK3CD, p70S6K	promoting
	Oesophageal squamous cell carcinoma	KLF-4, NF-κB	promoting
		HOXB13-mediated NF-κB/p65	promoting
miR-876-5p	Oesophageal squamous cell carcinoma	MAGE-A	promoting
miR-1299	Triple-negative breast cancer	MMPs	promoting
miR-135a	Bladder cancer	p21	Inhibiting

Table 1-9 CDR1 in Cancer

Extracted from Guo et al, 2020¹

1.12 Drug Resistant Mechanisms

The efficacy of treatment for recurrent OC declines rapidly with successive lines of therapy; median PFS declines from 6.4 months after the second relapse to 4.1 months after the fifth relapse (225) highlighting the need for effective therapies that delay disease progression or relapse (70).

1.12.1 Platinum resistance

Cisplatin and carboplatin are DNA damaging agents, with Carboplatin being a second generation platinum. They work by generating adducts/crosslinks with DNA purine bases, with a preference for guanine (226). While cisplatin is frequently cited in the literature, carboplatin is the drug of choice in the clinic as it has been shown to have similar efficacy and is less toxic (Section 1.1.7.2). These crosslinks cause damage that obstructs appropriate genome replication, transcription, and triggers cancer cell death. However, once the DNA is damaged, cells have mechanisms to try to repair this damage through DNA repair pathways (227).

1.12.2 Influx and efflux transporters

One resistance mechanism frequently used by OC cells is to actively pump these platinum compounds from the cells by upregulating efflux transporter, thus preventing DNA adduct formation (228). Platinum drugs enter cells via the influx transporter, copper transporter 1 (CTR1). This transporter is higher in cisplatin-sensitive A2780 OC cells compared to cisplatin-resistant A2780CP cells (229). Higher expression is correlated with increased influx of cisplatin. Treatment with cisplatin in A2780 cells resulted in rapid downregulation of CTR1, thereby inhibiting further cisplatin accumulation in the cells. This negative feedback loop has been proposed to result in resistance to cisplatin (230). The efflux transporter which removes platinum from the cell, namely ATPase copper-transporting alpha and beta (ATP7A and ATP7B), also play a role. The silencing of ATP7B in cisplatin-resistant cells (A2780-CP20 and RMG) resulted in increased sensitivity to cisplatin (231).

1.12.3 DNA Repair

As previously discussed, cells have a sophisticated process of repairing DNA lesions. Single-strand DNA lesions are repaired by NER, and double-strand lesions are repaired either by HR or NHEJ pathway. Once the platinum drugs have entered into the cytoplasm via CTR1, the platinum drugs enter the nucleus, where they form intrastrand and interstrand crosslinks with the DNA (226). Within the NER pathway, ERCC1-XPF and XPG endonucleases are recruited to cleave the DNA lesion i.e. repair the damage introduced by the platinum drug. High ERCC1 expression is associated with platinum resistance in epithelial OC but is not associated with patient survival (232).

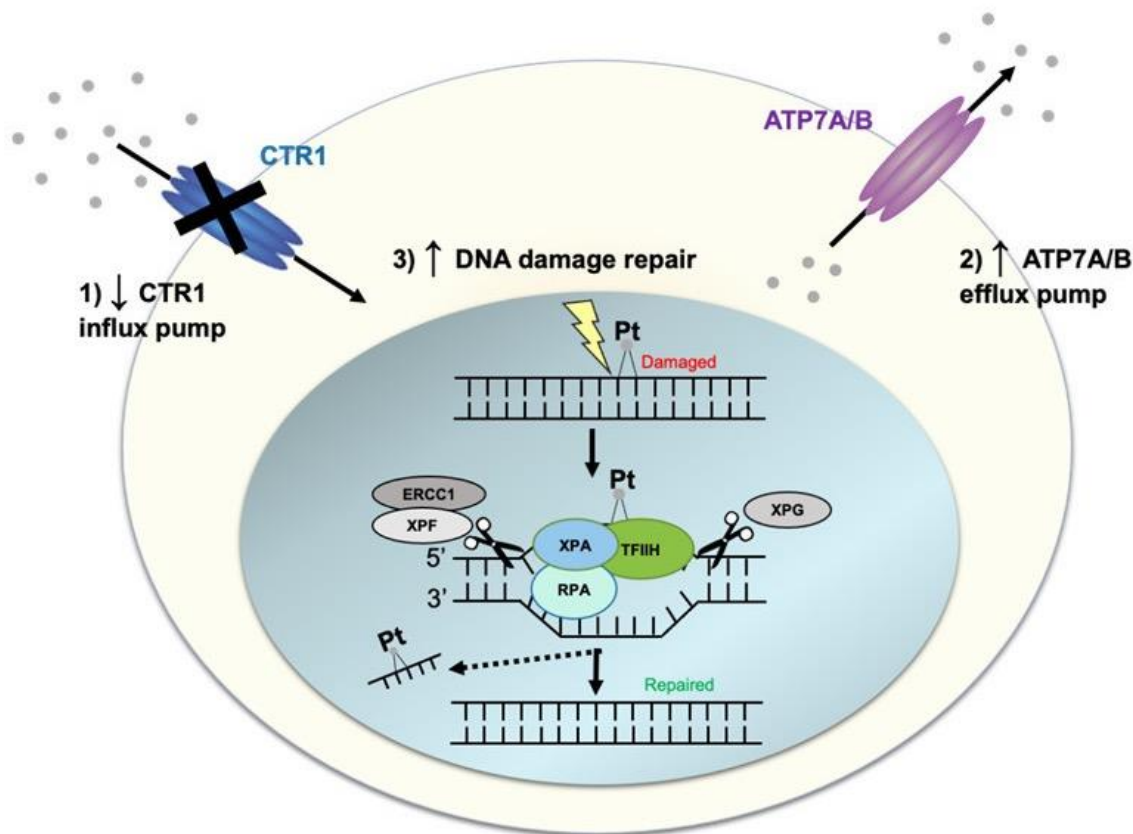


Figure 1-9 Schematic illustration of platinum resistance

Taken from Mechanisms of chemotherapy resistance in OC (226)

1.12.4 Taxol resistance

Paclitaxel is an anti-mitotic chemotherapeutic agent, which demonstrates effective antitumour properties against several cancers. It works by suppressing the microtubule dynamics by binding to the β -subunit of the tubulin heterodimer (233). However the development of multiple drug resistance mechanisms is a chief constraint to its success (234).

1.12.5 P-glycoprotein efflux channels

Like platinum agents, concentration of paclitaxel agents can be influenced by alterations in the efflux channels. Unlike platinum, it is the increase of P-glycoprotein (P-GP) efflux channels that results in increased removal of paclitaxel from the target cell (235).

1.12.6 Isoform Alteration

A second resistant mechanism can arise from microtubules altering their isoform, to promote lower stability and counteract the microtubule-stabilizing effect of paclitaxel (234). Paclitaxel resistant OC tumours are reported to have an increase in the proportion of β III isoform, indicating that upregulated production may confer paclitaxel resistance (116).

1.12.7 The PI3K/AKT pathway

The phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway is involved in drug resistance.

Typically this pathway becomes over-activated resulting in loss of function in PTEN (a negative regulator of PI3K/AKT), mutations in PI3K that confer constitutive activity, and hyperactivity of AKT(233). This results in the activation of pro-mitotic factors that override the anti-proliferative signals from paclitaxel exposure.

1.12.8 Upregulation of enzymatic pathways

In normal physiology, the Glutathione S-transferase P1 (GSTP1) enzyme detoxifies cells through a pathway controlled by PRMT6 activity, which inhibits the production of excessive GSTP1 precursor, G6PD (226). In this resistance mechanism, PRMT6 is downregulated in cancer cells, allowing for increases in G6PD production and higher levels of GSTP1 as a result. Thus, paclitaxel is mopped up by GSTP1 and detoxified in the cells before it can bind tubulin (226). Further, Cytochrome P450 (CYP) enzymes are responsible for the degradation of over 60% of Paclitaxel (114). While CYP enzymes are present in both healthy and malignant tissue, upregulation of these enzymes has been observed in cancerous tissue (236).

1.12.9 B-cell lymphoma 2 (Bcl-2) family

In the B-cell lymphoma 2 (Bcl-2) family, there are the pro-apoptotic factors, BAD and BAX, as well as the anti-apoptotic factors, Bcl-2, Bcl-XL, and Mcl-1 (228). Paclitaxel has been found to alter Bcl-2 activity, transforming it into a pro-apoptotic factor (237). Resistance to paclitaxel can be attributed to increases in anti-apoptotic Bcl-2 family members, as the anti-apoptotic factors inhibit FasL production, a ligand involved in cell death, by preventing its gene transcription. Further, genetic variances in Bcl-2 have been associated with varying rates of paclitaxel treatment success in patients. T>C variant (RefSNP rs1801018) in the Bcl-2 sequence is highly associated with paclitaxel resistance in OC (238).

Mechanisms of Paclitaxel Resistance

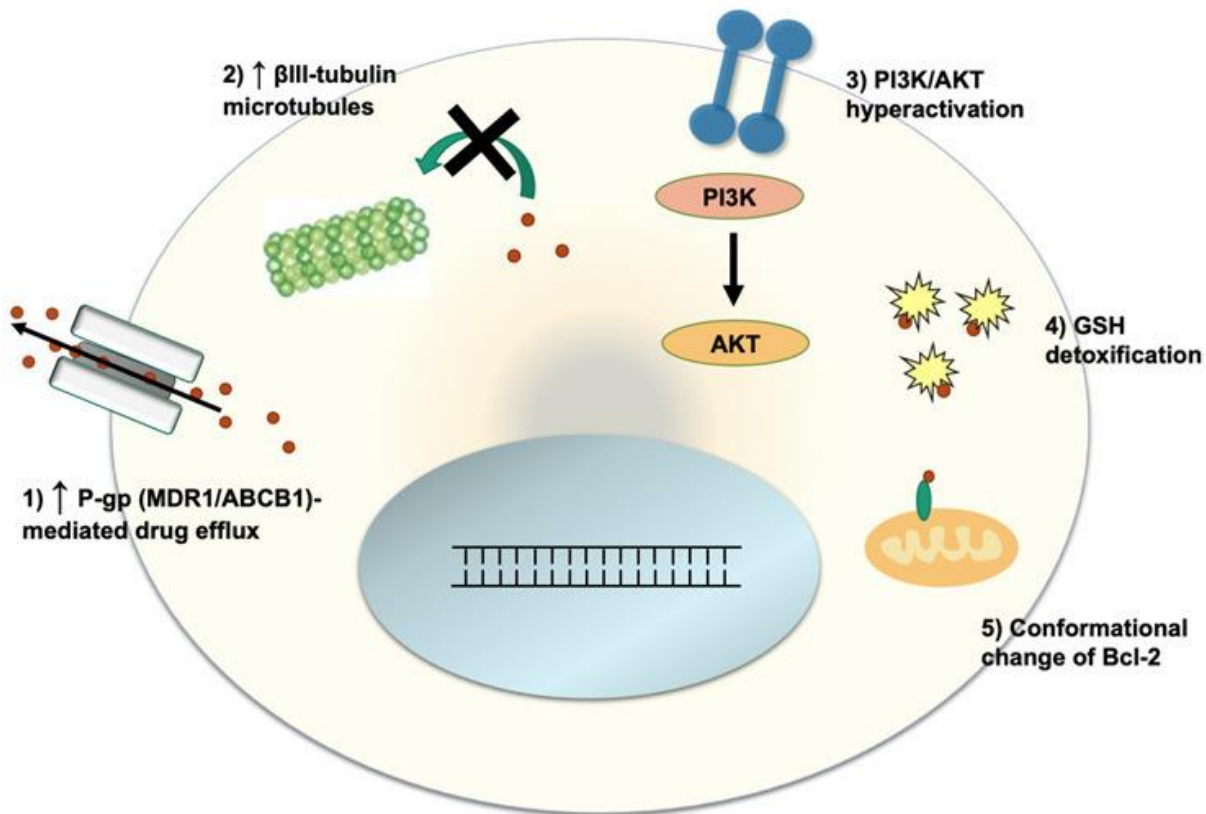


Figure 1-10 Schematic illustration of paclitaxel resistance.

Taken from Mechanisms of chemotherapy resistance in OC (226)

1.12.10 PARPi resistance

Resistance to PARPi's occurs in three main ways: restoration of homologous recombination, restoration of replication fork stability and drug target-related resistance(239).

1.12.11 Restoration of homologous recombination repair

It is understood that tumour cells with a compromised HRR system are most vulnerable to PARPi, through the mechanism in which the drug works. Consequently, a means in which tumour cells can become resistant to PARPi, is through restoration to an operable HRR system. Given the role *BRCA1* / *BRCA2* genes play in HRR, one way in which this is achieved, is through somatic reversion in a mutated *BRCA1/2* allele (240). Reversion mutations are somatic substitutions of base replacements, or insertions/deletions close to the main mutation of the gene, that restore the open reading frame of the gene and functional protein (241). They are typically close to the primary protein-truncating mutation and restore the open reading frame (ORF) of the gene and functional protein, switching the neoplastic cell from HRR deficient to proficient.

Reversion mutations as a mechanism of resistance have been demonstrated in PDX models, in vitro assays (242) and in ctDNA studies (243), in both *BRCA* genes and other HRR genes. In clinical studies

in *BRCA1/2*-mutated tumours, this restoration of the *BRCA1* or *BRCA2* gene functionality in HRR, is the most common acquired mechanism of resistance to PARPi's (244), and have been reported to occur in 20% to 40% of platinum-resistant or refractory diseases(245). Demonstrably, 22% of patients in the olaparib arm of SOLO3 clinical trial which assessed Olaparib vs. non platinum chemotherapy in patients with BRCA mutations in recurrent OC, had BRCA reversions detected in their ctDNA at disease progression **Figure 1-11** extracted from ESMO E-Learning: Management of BRCA-Mutated OC – An Update (246)). No responses to olaparib were seen for patients with BRCA reversions identified at baseline (4%). In SOLO3, PFS was improved with olaparib over chemotherapy however this did not translate into OS. Similarly, patients in the Ariel2 trial, treated with Rucaparib, had significantly longer rucaparib PFS in those without BRCA reversion mutations detected in pretreatment cfDNA than those with reversion mutations (median, 9.0 vs. 1.8 months; HR, 0.12; $P < 0.0001$)(243). Interesting, in no cases were reversion mutations identified in cfDNA that did not associate with concurrent or imminent progression, suggesting that these findings warrant a change in therapy. Thus, reversion mutations seem to be a well-validated mechanism of resistance to PARPi (247). It is further hypothesized that *BRCA* reversion mutations could contribute to worse outcomes in OC due to innate resistance to subsequent therapies driven by reversion mutations in *BRCA* (245).

22% of patients in the olaparib arm of SOLO3 had *BRCA* reversion mutations detected in their ctDNA at disease progression³

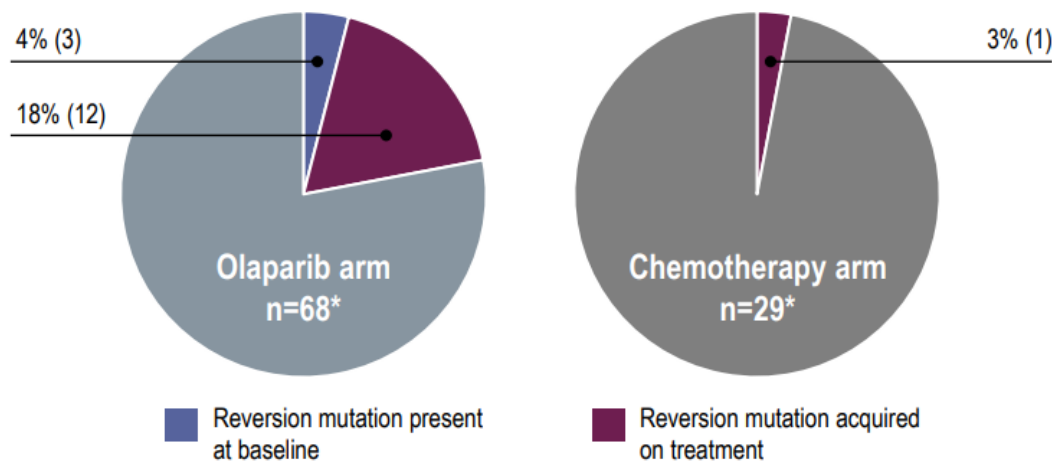


Figure 1-11 Prevalence of *BRCA* reversion mutations in SOLO3 Trial

1.12.12 Restoration of replication fork stability

An alternative way in which tumour cells acquire resistance to PARPi is through adopting mechanisms for protecting the replication fork. The PARPi resistant tumour may still have an HR defect, but will not display a cytotoxic effect from PARPi exposure(240). In these scenarios, there is often an upregulation of the ATR/CHK1 pathway, thereby activating the phosphorylation of multiple proteins contributing to fork stability. *BRCA1* cells have demonstrated resistance to PARPi by reducing the recruitment of the nuclease, MRE11, to the stalled fork, thereby resulting in fork protection (248).

1.12.13 Drug target-related resistance

PARPi function through the blocking of PARP enzymes, leading to a decline in effectiveness of DNA single stranded repair, which is most effective in a tumour cell whose HRR system is also defective (249). Therefore, another mechanism of PARPi resistance may be decreased expression of PARP enzymes. In vitro studies have shown that PARP1 levels can be lower in cell lines that have acquired resistance to the PARPi (250). Fascinatingly, a PARP1 mutation (1771C > T) has been identified in an OC patient who demonstrated de novo resistance to olaparib.

1.12.14 Prioritising alternative DNA repair pathways

A number of studies have shown that suppression of NHEJ activity in HR-deficient tumours can restore the HR activity and regulate PARPi resistance (247). *BRCA1* is important for restoring 53BP1 to DNA ends to facilitate HR repair, however in *BRCA* defective tumour cells, this process cannot complete. By restoring the loss of 53BP1 DNA end resection and rescued the HR defects (247, 251).

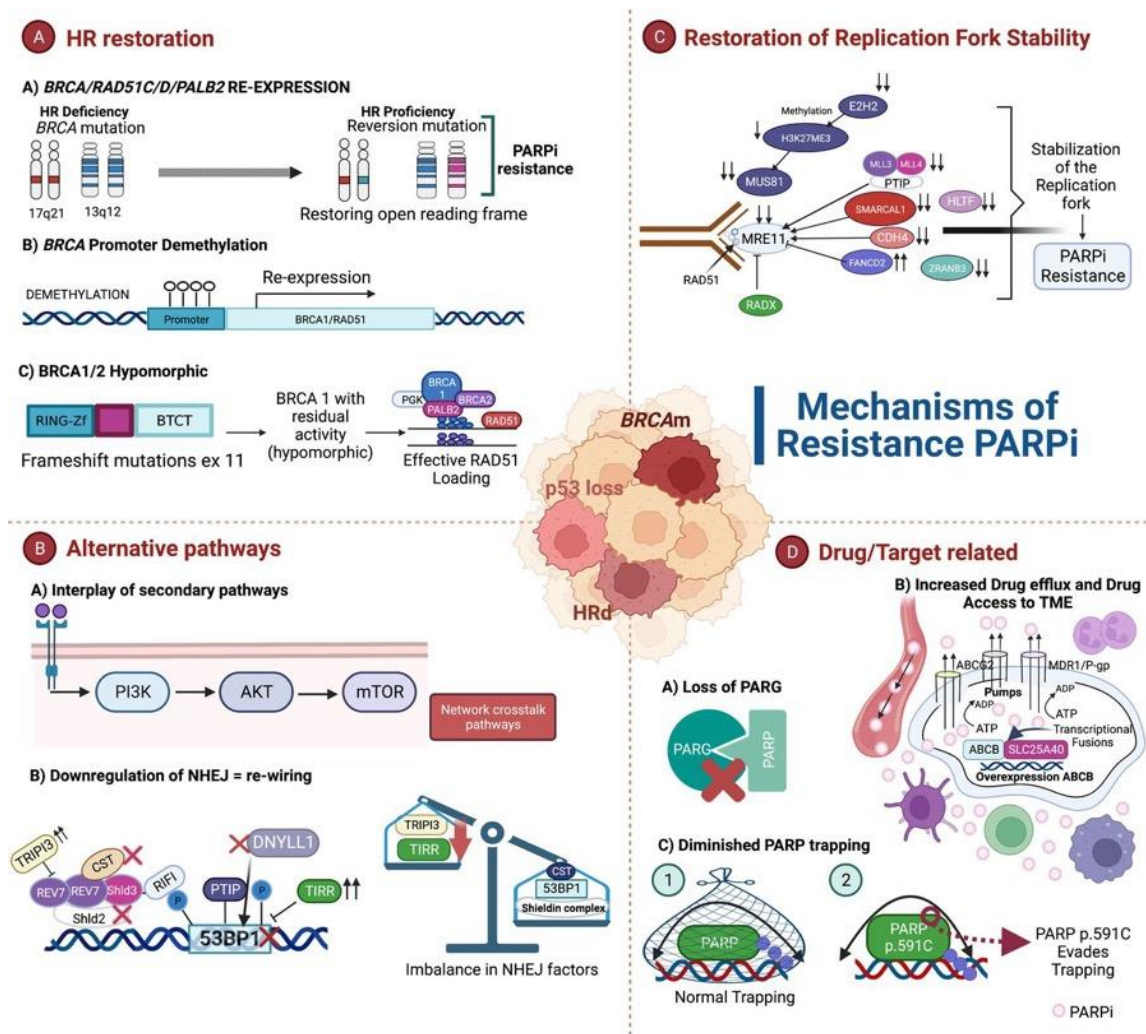


Figure 1-12 Schematic illustration of PARPi resistance

Taken from Overcoming PARPi resistance in OC (251)

1.12.15 Markers of Stemness

Stemness markers are genes that regulate self-renewal and pluripotency, reflecting the ability of cancer stem cell populations to sustain tumour growth and resist therapy(252). Markers of stemness such as *NANOG*, *SOX2*, *OCT3/4*, *ALDH1A1* and *NOTCH* have been implicated in driving resistance to chemotherapy and PARPi in OC (253, 254). Their expression contributes to the persistence of cancer stem cell populations and has been linked with poor clinical outcomes, underscoring their relevance as potential biomarkers and therapeutic targets.

1.13 Aims and Objectives

1.13.1 General Aims

The broad aims of this thesis are to describe the epidemiological, demographic, and genetic characteristics of OC in Ireland and to identify the distinct traits and molecular properties within three *BRCA* subtypes in OC - *BRCA* wild-type, *BRCA1* mutant, and *BRCA2* mutant - specifically in relation to circRNA.

1.13.2 Hypothesis

The central hypothesis of this thesis is as follows:

- There is a distinct epidemiological pattern of OC in Ireland
- Traits and molecular properties vary across different *BRCA* statuses
- Specific acquired resistance mechanisms existing in OC that are unique to each *BRCA* status.
- circRNA profiles associated with resistance may serve as (a) predictive biomarkers for defining such resistance and (b) functional elements in the mechanisms of resistance.

1.13.3 Specific Objectives

The hypothesis will be tested through the following specific objectives, encompassing demographic examination, in silico analysis, and experimental and translational work:

- I. To report the genetic and clinico-pathological characteristics of women in Ireland with OC, specifically the prevalence of *BRCA* mutations in HGSOC, and the characteristics of young women diagnosed with OC
- II. To undertake in silico analysis of pertinent miRNA in OC, with subsequent validation in appropriate OC cell lines
- III. To recruit women with OC with an identified *BRCA* status to facilitate an in-vivo validation study of circRNA identified in the experimental phase.
- IV. To profile OC cell lines, pinpointing circRNA signatures specific to *BRCA* status, and validate these findings in patient samples.
- V. To develop OC resistant cell lines with and without *BRCA1* and *BRCA2* mutations for clinically relevant therapies (carboplatin, paclitaxel and olaparib), and identify novel circRNAs associated with each that can potentially be tested for their utility as candidate predictive biomarkers for the development of acquired resistance.
- VI. A subset of the identified circRNAs will also be tested to see if they are functionally involved with drug resistance.

Materials and Methods

Chapter 2.0

2 Materials and Methods

2.1 Epidemiology Analysis Methodology

2.1.1 Ethical Approval

Ethical approval was obtained from the relevant bodies, namely the SJH/TUH Joint Research Ethics Committee (Project ID: 3611) and the Beaumont Hospital Ethics (Medical Research) Committee (REC REF 23/51), to conduct a retrospective analysis on the prevalence of *BRCA* PVs in OC in an Irish cohort. For the project titled “Reporting the Characteristics of Young People with OC in an Irish Cohort”, patients had previously provided consent to participate in the Gynaecological Disease Research Biobank at St James’s Hospital. This study was approved as an amendment of the Gynaecologic Biobank (Submission Number: 5594) by the SJH/TUH Joint Research Ethics Committee Ethics Review Board. Further ethical approval was obtained for the CAMPAIGN registry under Project ID 3824, with a subsequent amendment under the same project ID to approve the conduct of a PPI survey.

2.1.2 Study of prevalence of BRCA CAVs in an Irish cohort

An inclusion criteria was clearly defined for this retrospective study, which was conducted from January 1, 2020, to December 31, 2023. The study included patients diagnosed with HGSOC, HGEOC, primary peritoneal carcinoma (PPC), or fallopian tube carcinoma (FTC) who were referred to two national molecular diagnostic laboratories (St. James's Hospital, Dublin, and Beaumont Hospital, Dublin) for tumor *BRCA* and/or germline *BRCA* (g*BRCA*) testing. Testing was performed upon physician request. Data collected for the study included patient age, diagnosis, testing outcomes, referral center, and various quality metrics.

2.1.3 Study of clinco-pathological characteristics of young people diagnosed with OC in Ireland

This study employed a retrospective cohort study design to investigate various factors associated with outcomes in young people with OC. Patients diagnosed with invasive OC under the age of 50 between January 2010 and September 2023 were included based on their identification through the Gynecologic BioBank at St. James's Hospital, for which they had previously given consent to participate. The inclusion criteria required patients to have been diagnosed with invasive OC and be younger than 50 years at diagnosis. Data collected included tumor subtype, tumor grade, stage, age, parity, menopausal status, genetic characteristics, and survival outcomes. The patients' demographic details, clinical presentations, histological features, treatments, and survival outcome data were primarily gathered through the biobank data and by reviewing patient electronic medical records. When necessary, inpatient and outpatient paper charts were also examined.

2.1.4 BRCA1/2 CAVs Detection

A *BRCA1/2* smMIP (single-molecule molecular inversion probe) panel for the detection of single

nucleotide variants and short insertions and deletions was employed, as described by Neveling et al (255) . Sequencing was completed using 2x150bp sequencing on an Illumina NextSeq 550 Dx instrument. This assay, applied to peripheral blood and FFPE derived DNA, enables detection of both somatic and germline mutations. Furthermore, BRCA1/2 MLPA (Multiplex ligation-dependent probe amplification) was utilized for large genomic rearrangement detection in peripheral blood samples, utilizing CE-IVD MLPA probe mixes (MRC-Holland). These methodologies ensured comprehensive screening for BRCA1/2 mutations. This was performed by the Cancer Molecular Diagnostic (CMD) Laboratory, St James Hospital, Dublin 8.

2.1.5 Variant Classification

Variants detected during NGS underwent meticulous classification following guidelines provided by the American College of Medical Genetics and Genomics (ACMG) for rigorous and standardized categorization (256). Additionally, variant classification adhered to proposed guidelines by the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) consortium, as per recommendations by the International Agency for Research on Cancer (IARC). Per standard reporting, classification included five classes: Benign (Class I), Likely Benign (Class II), Variant of Uncertain Significance (VUS, Class III), Likely Pathogenic (Class IV), and Pathogenic (Class V). Variants classified as Likely Pathogenic or Pathogenic (Classes IV and V) were considered Clinically Actionable Variants (CAV) and are reported collectively in our analysis. VUS (Class III) are also reported separately for completeness but were not considered clinically actionable.

2.1.6 Statistical Analyses

Descriptive statistics were employed to summarize demographic and genetic characteristics of the study population, encompassing patient age, test results, and the presence of *BRCA* clinically actionable variants (CAVs). Numerical variables in the patient cohort were described in terms of the median (and range) and compared using the student's *t* test. Data analysis utilized Python (v.3.8) programming language and the scipy (v1.10.1) library, facilitating efficient and comprehensive analysis of collected data.

2.2 In Silico Analysis Pipeline

2.2.1 KM Plotter

KMplotter, is a web-based tool (<https://kmplot.com>) that enables survival analysis across multiple cancers and datasets. KMPlot functions by downloading OS data from databases such as GEO, EGA and TCGA (257). The obtained Kaplan-Meier survival curves and associated statistics provided valuable insights into the relationship between mRNA/miRNA expression and cancer prognosis. The miRNA were split into two groups per auto-selection of the best cutoff for each gene, in order to assess its prognostic value. Kaplan-Meier survival plot, and the hazard ratio with 95% confidence intervals and log-rank P value were calculated. miRNAs with a False Discovery Rate (FDR) of 100% were omitted from further evaluation, as where miRNA flagged as having very low median expression by the online tool. Lastly the miRNA where only included if they were deemed to be statistically significant $p < 0.05$. Statistical significance is indicated in the results as follows, as per 2024 GraphPad Software: Nos (not significant, $P > 0.05$), * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), and **** ($P \leq 0.0001$). N/A indicates that there was not sufficient information available.

2.2.2 Target Scan

TargetScan employs sophisticated algorithms to predict miRNA binding sites within mRNA sequences, aiding in the identification of potential miRNA-gene regulatory interactions (258). The selected gene signatures of miRNA was entered into the online tool (available at https://www.targetscan.org/vert_80/), and filtered for human species. A table of predicted target genes associated with each miRNA was subsequently generated.

2.2.3 cBioportal

cBioPortal is a web platform that enables interactive exploration of multidimensional cancer genomics data, allowing users to visualize and analyze genetic alterations, gene expression, and clinical outcomes across various cancer types (259-261). Selected genes were inputted into the cBioportal platform to give gene associated survival outcomes.

2.3 Preparation and handling of materials

Reagents and chemicals used in the laboratory were of analytical grade and stored in accordance with manufacturers' instructions. All chemicals and waste were disposed of according to local guidelines. Chemicals were purchased from Merck KGaA (Darmstadt, Germany) or Sigma-Aldrich (MO, USA) unless stated otherwise.

2.3.1 miRNA validation

2.3.1.1 miRNA Primer optimisation

Optimization of miRNA amplification through endpoint PCR was conducted by varying annealing temperatures and cycling conditions to determine the most efficient parameters for specific miRNA detection. Different annealing temperatures were tested, typically ranging from 55°C to 65°C, to identify the temperature that provides the highest specificity and yield of the target miRNA. Additionally, the number of PCR cycles was adjusted, with variations in both the denaturation and extension times, to optimize the amplification process. By systematically altering these conditions, the optimal parameters were identified, which minimized nonspecific amplification and maximized the efficiency of miRNA detection. The results from these optimizations were used to refine the PCR protocol, ensuring robust and reproducible amplification of the target miRNAs for downstream analysis.

2.3.2 cDNA synthesis for miRNA

Using the SRNA Primer Design tool available at <http://www.srnaprimerdb.com/design>, miRNA primers (miRNAPrimer) were designed to validate miRNAs identified from in silico analyses (**Figure 2-1**). The linear poly(A) tailed RT-PCR method was used, whereby RNA was extended by a poly(A) tailing reaction using poly(A) Polymerase and ATP. The sncRNA with a poly(A) tail converts into cDNA through reverse transcription primed by a universal linear poly(T) RT primer, and then PCR-amplified using a specific forward primer and a universal reverse primer. The RT-PCR amplification can be monitored by real-time detection or by end-point detection for quantifying the sncRNA transcript level. The PCR amplicons created, are then sequenced for validating the expression of the specific sncRNA.

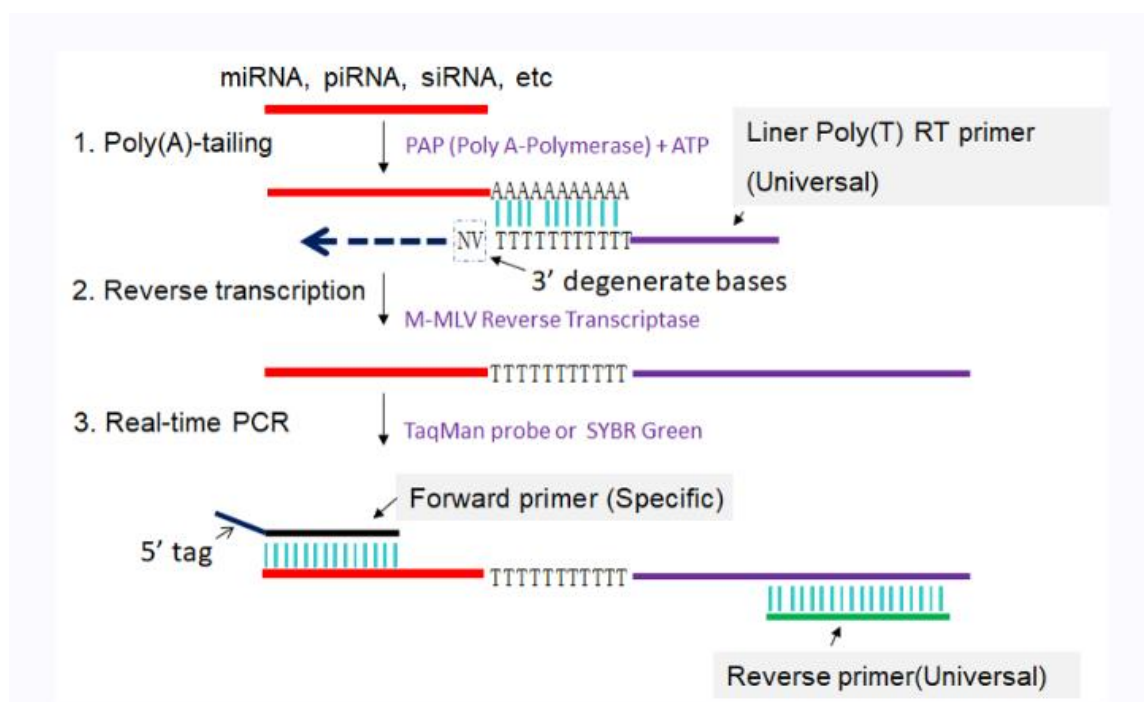


Figure 2-1 Scheme for linear poly(A) tailed RT-PCR

Initially, a mix of RNA (1.0 μ g), RT-Primer (2 μ L) and molecular grade H₂O (0.5 μ L) was prepared and incubated at 65°C for 5 minutes, then placed on ice for 1 minute. Next, a reaction master mixture was then prepared for each reaction, containing 4 μ L of 5X RT buffer, 2 μ L of 10X PAP buffer, 4 μ L of dNTPs (10 mM each), 0.8 μ L of RNase inhibitor (40 U/ μ L), 1.2 μ L of RTase (200 U/ μ L), 5 μ L of MgCl₂ (25 mM), 0.5 μ L of ATP (100 mM), and 1 μ L of PAP (2 U/ μ L). This reaction mixture was added to the RNA/primer mixture, mixed briefly, and allowed to sit at room temperature for 2 minutes. The samples were then incubated at 37°C for 30 minutes in a PCR machine, followed by heat inactivation at 95°C for 5 minutes and immediate cooling on ice. The synthesized first-strand cDNA was stored at -20°C as a 10- or 20-fold dilution until further use in real-time PCR experiments.

2.3.3 Real-Time qPCR

miRNA validation was carried out using quantitative PCR (qPCR). The PCR reaction mixture comprised 12.5 μ L of Syber (Promega, WI, USA), 0.5 μ L of forward primer (10 μ M), 0.5 μ L of reverse primer (10 μ M), and 10.5 μ L of DNase-free H₂O (Sigma-Aldrich), resulting in a total reaction volume of 24 μ L. To this mixture, 1 μ L of cell culture supernatant was added. The qPCR was conducted over 40 cycles using the 7500 Fast Real-Time PCR System (Applied Biosystems). The cycling conditions are outlined in **Table 2-1**.

Cycling step	Temperature (°C)	Time	Number of cycles
Initial denaturation	95	10 mins	1
Denaturation	95	15 secs	40

Annealing	60	1 min	
Melting curve	95	15 secs	1
	60	1 min	
	95	30 secs	

Table 2-1 qPCR conditions.

2.3.4 miRNA Statistical Analysis

Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by a post hoc test to identify specific group differences. A probability threshold of $p < 0.05$ was set to indicate significant differences between groups. The significance levels were categorized as follows: not significant (ns, $P > 0.05$), * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), and **** ($P \leq 0.0001$).

2.4 Cell Line Authentication

2.4.1 STR Profiling

Genomic DNA was extracted from cell lines as per section 2.9.4, and prepared according to Eurofins Genomics STR profiling specifications: > 20 ng/μL in total volume of > 20 μL. Genetic characteristics were determined by PCR-single-locus-technology. Cell line analysis was performed in comparison with the online database of the DSMZ (262) and the Cellosaurus database (263). STR profile was queried using the following STR criteria: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, AMEL, D5S818, FGA, D19S433, vWA, TPOX and D18S51. This was repeated post development of resistant models.

2.4.2 BRCA Status Verification

Genomic DNA was extracted from cell lines as per section 2.9.4, and sent to the Cancer Molecular Diagnostics Laboratory (St James's Hospital) for *BRCA* profiling using smMIP probes, which were designed using MIPgen (264). Briefly, 100ng DNA were combined with 1μL of the *BRCA* smMIP pool and 7μL of the Hybridisation mastermix was prepared in-house according to previously published protocols (264). This mixture was incubated as follows on a thermocycler: 95°C for 9 min, 8 X (95 °C for 1 min, 60°C for 150 min), 4°C hold. Following hybridisation, 2μL of Exonuclease mastermix was added to each sample and incubated at 37°C for 45min followed by inactivation at 95°C for 2min. Samples were then barcoded by adding 5μL of the appropriate barcode and 25μL of iProof mastermix (Bio-Rad Laboratories, Hercules, CA, USA) followed by incubation at 98°C for 30s, 22 X (98°C for 10s, 60°C for 30s, 72°C for 30s), 72°C for 2 min, 4°C hold. Libraries were pooled in groups of 8 and purified using Ampure XP beads (Agencourt) according to the manufacturer's instructions. Pooled libraries were quantified using Qubit (ThermoFisher) and sequenced on a NextSeq 550Dx (Illumina) using custom sequencing primers according to the manufacturer's instructions. Resulting FASTQ files were then analysed using SeqPilot (JSI Medical Systems, Germany)(265).

2.5 Cell Culture

Cell culture was performed aseptically in accordance with good laboratory practice in an ABS optimale 18 laminar air flow unit (LAF) (Aulnay Sous Bois, France). The LAF was exposed to UV light for approximately 30 min, and subsequently sanitised using 70% absolute ethanol pre and post use. Cell culture reagents were warmed to 37°C prior to use, unless stated otherwise. All cells were cultured in vented tissue culture treated flasks (Sarstedt) and maintained at 37°C with 5% CO₂ in a humidified atmosphere.

2.5.1 Cell Culture Reagents

RPMI-1640 and Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and L-glutamine were purchased from Sigma-Aldrich. All media were supplemented with 10% FBS, unless stated otherwise. DMEM was additionally supplemented with 0.04 µM Sodium pyruvate (Sigma-Aldrich).

2.5.2 Cell line propagation

Cells were removed from long term storage and thawed rapidly at 37°C for 2 min. Once thawed, the cells were mixed gently and added to 5 mL pre-warmed complete media in a T25 cm³ tissue culture flask.

2.5.3 Cell line subculture

Cells were visualised daily using an inverted phase-contrast microscope (Nikon Corp., Tokyo, Japan). Sub-culturing was performed when cell cultures reached approximately 80% confluency. Cell culture medium was decanted, and the cells were washed with 3 mL 1X PBS (HyClone™, MA, USA). Two mL trypsin ethylene-diamine tetra-acetic acid (EDTA) (Sigma-Aldrich) was added to the flasks. Flasks were incubated at 37°C for approximately 3 min to allow the trypsin to detach the cells from the flask surface. Three mL complete medium was added to the flasks to inactivate the trypsin, cells were dislodged from the surface of the flask, transferred to a sterile 15 mL tube and pelleted by centrifugation at 1300 rpm for 3 min in an Eppendorf™ 5804 centrifuge (Eppendorf). The supernatant was discarded, and the cell pellet re-suspended in 10 mL complete medium. This suspension was used to seed fresh T75 cm³ flasks at several different densities.

2.5.4 Cryopreservation

Cells were trypsinised (as per section 2.1.2.4). Resulting pellets were re-suspended in Cellbanker® cryopreservation media (AMS Biotechnology, Abingdon, UK) at a concentration of 1-1.5x10⁶ cells/mL. Aliquots were stored at -80°C.

2.5.5 Agarose gel electrophoresis

A 2% agarose gel (Sigma-Aldrich) was prepared in 100 mL 1X Tris-acetate EDTA (TAE) buffer (Sigma-Aldrich). The agarose was dissolved by heating in a microwave for 2-3 min. Five µL SYBR Safe DNA gel stain (Invitrogen, CA, USA) was added to the solution and the mixture was poured into a gel tray with

well forming combs and allowed to set. Once set, the comb was removed, and the gel was covered with 1X TAE buffer. Six μL appropriate ladder (Fisher Scientific) was added to the first well and 15 μL sample was added to subsequent wells. If required, samples were mixed with 2 μL 6X loading dye (Fisher Scientific) before loading on to the gel. Gels were electrophoresed at 120V for 60 min. Gels were visualised using a Bio-Rad (CA, USA) gel imaging system.

2.5.6 Antimicrobial Use

Cells were maintained without antimicrobials to avoid confounding effects on drug-response phenotypes. Antimicrobials can alter mitochondrial function, stress signalling and drug transporter activity (266), thereby influencing sensitivity to anti-cancer therapies. Their presence may also mask low-level contamination, introducing uncontrolled variability (267). To minimise this risk, strict aseptic technique was adopted, and cultures were routinely screened for mycoplasma contamination every 12 weeks. This schedule is consistent with best practice guidelines, which recommend periodic mycoplasma monitoring as a quality control measure in long-term cell culture (268).

2.5.7 Mycoplasma testing

Cells were tested for mycoplasma infection within 4 weeks of receipt and every 16 weeks thereafter. At approximately 80% confluence, the supernatant was removed from T75 cm^3 flasks. The supernatant was centrifuged at 300 $\times g$ to remove cellular debris and a PCR based reaction, adapted from Young *et al* (269) was used to determine the presence or absence of mycoplasma. The primers were designed to encompass common mycoplasma species (**Appendix 1**). The PCR reaction consisted of: 12.5 μL Green 2X GoTaq (Promega, WI, USA), 0.5 μL FWD primer (10 μM), 0.5 μL REV primer (10 μM) and 10.5 μL DNase free H_2O (Sigma-Aldrich). Total reaction volume was 24 μL , which included 1 μL cell culture supernatant. PCR was performed over 40 cycles using the 7500 Fast Real-Time PCR System (Applied Biosystems) with the cycling conditions as shown in **Figure 2-2**.

Temperature	Time
95°C	5 min
94°C	30 sec
55°C	30 sec
72°C	1 min
72°C	10 min



40 cycles

Figure 2-2 Cell Line Preparation PCR conditions for mycoplasma testing

The resulting PCR products were electrophoresed on a 2% agarose gel. A PCR negative control and a known mycoplasma positive control were included. A band at 270bp was indicative of a positive result.

2.5.8 Cell Counting

Cell counting was performed using two approaches depending on the experimental setup. Cells were

seeded at defined densities and subsequently counted using either a bright-line hemocytometer (Hausser Scientific, PA, USA) in combination with Trypan Blue (GIBCO) (0.4% v/v) for cell counting and viability assessment, or with disposable hemocytometers (C-CHIP, NanoEntek, MA, USA). Cells were pelleted as above (as per section 2.1.2.4) and re-suspended in a specific volume of media until a single cell suspension was obtained. A 20 μ L aliquot of suspension was added to 180 μ L Trypan Blue and mixed gently. The haemocytometer was cleaned using 70% EtOH and a glass cover slip was placed over the chamber. The edge of the cover slip was touched gently by a pipette tip and the chamber filled by capillary action. Viable cell number per mL was calculated using the following equation:

Average no. of cells $\times$ 10,000 $\times$ 10 = no. of cells/mL, where 10,000 accounts for the μ L volume under the cover slip and 10 equals the dilution factor.

2.6 Developing and Characterising Resistant Cell Line Models

2.6.1 Drug Treatment

During the drug treatment phase, doses were carefully calculated based on established pharmacokinetic data (**Appendix 7**) and specific experimental objectives. Each drug was then added to the cell culture media at the designated concentration, ensuring thorough mixing for uniform distribution. Vehicle control wells were treated with the corresponding solvent at equivalent volumes. After a 24-hour exposure period, the media containing the drug was removed to prevent prolonged exposure. This removal was followed by washing the cells with PBS, after which fresh media was added. Following the drug treatment, a recovery period was observed. During this time, the cells were closely monitored for signs of recovery, which included assessing cell morphology, viability, and proliferation rates. Once the cells showed signs of full recovery, indicated by a return to normal morphology and growth patterns, they were retreated with an incrementally increased dose of the drug.

2.6.1.1 Carboplatin Treatment

Carboplatin was purchased from Bio-Techne Ireland Limited, dissolved in sterile water at a concentration of 10 mM and stored at -20°C. Conventional doses of carboplatin administered to patients are generally aimed at giving an AUC value of between 5 and 7 mg/mL/min (270, 271). Pharmacokinetic studies have reported that after intravenous bolus injection of 375 mg/m² carboplatin peak-plasma levels of ~39 μ g/mL are achieved, which drops to 9 μ g/mL within 2 h (272). Knowing this, and that Carboplatin has a molecular weight of 371.25 Da (273), this is equivalent to 24.24 μ M.

2.6.1.2 Paclitaxel Treatment

Paclitaxel was purchased from Merck Life Science Limited, dissolved in ethanol at a concentration of 100 μ M and stored at -20°C. Paclitaxel is usually administered at a dose of 175 mg/m² in the doublet

treatment regime(271). Pharmacokinetic studies for this dose show peak-plasma concentrations as high as 10,000 ng/mL but drop off quickly after 24 h to 50 ng/mL and below(274). Given Paclitaxel has a molecular weight of 853.91 Da (275), this is equivalent to 58.55nM.

2.6.1.3 Olaparib Treatment

Olaparib (AZD2281) was gifted from AstraZeneca pharmaceuticals, dissolved in DMSO at a concentration of 10 mM and stored at -20°C. The maximum tolerated dose of olaparib is 400 mg twice daily, though in clinic 300mg twice daily is used. Absorption is rapid and a single-dose olaparib 300mg tablet led to peak plasma exposures after around 2 h (276). Peak-plasma values for a single dose of 400 mg olaparib have been reported as ~7 µg/mL (276, 277). It is excreted rapidly and drops to less than 0.1 µg/mL after 50 h (277). For a dose of 400 mg administered twice daily for 15 days, peak-plasma concentrations were found to be similar (278). Given that Olaparib has a molecular weight of 434.46 Da (279), this is equivalent to 16.11µM. DMSO was used as the vehicle control for PARPi experiments, whereas an untreated control was maintained as the vehicle control for other drugs.

2.6.2 Developing a Dose for Resistance Model Creation

3500 cells of COV362 and 4000 cells of OVSAHO were seeded into flat-bottomed 96-well microtitre plate (Nunc, Sigma-Aldrich, UK) in 100µl of media per well at a cell density of 1.1x10⁵ cells/ml. After 24hs the wells were treated with 4 different doses of Carboplatin (20, 40, 60 and 80 µM), Paclitaxel (50, 100, 150, 200 nM), Olaparib (10, 20, 40 and 80 µM) and Carboplatin / Paclitaxel combination (20/50, 40/50, 20/100, 40/100 µM / mM). DMSO was used as vehicle control. The plates were incubated at 5% CO₂ at 37°C.

2.7 Cell Viability and Proliferation Assays

2.7.1 Resazurin assay

Cells were seeded at 1 x 10³/ well in a final volume of 100 µL/ well in 96-well plates. Cells were treated with appropriate drug for 24 hrs. After 24 hrs, 20 µL/well of a 1:1000 dilution of Resazurin labelling solution (Sigma-Aldrich), final concentration – 10 µM, was added into each well and incubated for 4 h at 37°C. Following incubation, fluorescence was measured on a Varioscan Lux Plate Reader (ThermoScientific) with reference wavelength set to Excitation 560 nm, Emission 590 nm. Control wells of seeded cells that were untreated with drug were also run and set to 100%. Plates were run with Resazurin assay in SKANT reader at pre-determined time points post treatment (24 h, 48 h and 72 h).

2.7.2 Cell Counting Kit (CCK-8) assay

The CCK-8 (Cell Counting Kit-8) assay (CCK-8 reagent) contains a tetrazolium salt-based solution. The CCK-8 reagent is reduced by cellular dehydrogenases in metabolically active cells, resulting in the formation of a water-soluble formazan dye. This dye exhibits an absorbance peak at around 450 nm,

which is directly proportional to the number of viable cells in the culture. Cells were seeded at a cell density of 3×10^3 / well in a final volume of 100 μ L/ well in 96-well plates. Following culture, 10 μ L/well of CCK-8 reagent was added into each well and incubated at 37°C. Absorbance was measured at various time intervals (2 hr - 72 hr) on a Varioscan Lux (ThermoScientific) Plate Reader with reference wavelength set to 450 nm. Control wells of seeded cells that were not treated were also run and set to 100%.

2.7.3 Bromodeoxyuridine (BrdU) incorporation assay

A 10 mM BrdU stock solution was prepared by dissolving 100 mg BrdU in 32.5 mL of anhydrous DMSO. For labeling, a 10 μ M BrdU solution was made by diluting 10 μ L of stock in 10 mL of pre-warmed tissue culture medium. Cells were seeded at 3×10^3 cells/well in 96-well plates and treated with the BrdU solution for 2 hours at 37°C. After washing with PBS, cells were fixed with 3.7% formaldehyde, permeabilized with Triton X-100, and subjected to acid washes to denature DNA. After neutralization, cells were incubated overnight with anti-BrdU primary antibody and then with a fluorescent secondary antibody. Imaging was performed to detect BrdU incorporation, and signal intensity was measured using a Varioscan Lux Plate Reader to quantify cell proliferation. Control wells with non-proliferating cells were included for baseline measurements

2.8 Setting Up Clinical Model

Three 6 well plates were set up with 100,000 cells per well in 2mls of appropriate media for each cell line. Flasks were incubated at 37°C for 24 h and after 24hs the wells were treated with a range of drug doses for each treatment arm. Carboplatin (6, 12 and 24 μ M), Paclitaxel (15, 30, and 60 nM), Olaparib (16, 32 and 64 μ M) and Carboplatin / Paclitaxel combination (6/15, 12/60, 24/60 μ M / nM) were added to the selected wells. These were treated for 24 h in the case of carboplatin and paclitaxel, and 72 h for Olaparib. DMSO was used as a vehicle control and an untreated well was maintained.

2.9 RNA/DNA isolation and quantification

2.9.1 RNA Isolation

2.9.2 RNA Isolation using Qiagen miRNeasy kit

RNA isolation for miRNA experiments was conducted using the miRNeasy Mini Kit (Qiagen, Sussex, UK). The cell culture supernatant was removed, and cells were lysed in the culture flask with 2 mL of QIAzol lysis reagent (Qiagen). Cells were dislodged using a cell scraper, and the lysate was mixed thoroughly with a pipette tip. For phase separation, 140 μ L of chloroform (Sigma-Aldrich) was added, and the sample was shaken vigorously for 15 seconds, followed by a 3-minute incubation at room temperature. The samples were centrifuged at $12,000 \times g$ for 15 minutes at 4°C, and the upper aqueous phase containing RNA was carefully transferred to a new tube. RNA precipitation was performed by adding 525 μ L of 100% ethanol (Sigma-Aldrich) to the aqueous phase and mixing well.

A volume of 700 μL of this mixture was pipetted onto the center of an RNeasy mini spin column and centrifuged at $8,000 \times g$ for 15 seconds. The flow-through was discarded.

To remove genomic DNA, 350 μL of RW1 buffer was added to the spin column, and centrifugation was repeated with the flow-through discarded. A DNase I solution was prepared by mixing 10 μL of DNase I with 70 μL of buffer RDD, inverted to mix, and applied to the column membrane, followed by a 15-minute incubation at room temperature.

Afterward, 350 μL of RWT buffer was added, and the column was centrifuged for 15 seconds at $8,000 \times g$. Next, 500 μL of RPE buffer was added for washing, followed by a second wash with another 500 μL of RPE buffer and centrifugation for 2 minutes at $8,000 \times g$.

To eliminate residual RPE buffer, the spin column was placed into a fresh 2 mL collection tube and centrifuged for 1 minute at maximum speed. The column was then transferred to a new 1.5 mL collection tube, and 30-50 μL of RNase-free water was added directly to the membrane. This assembly was centrifuged for 1 minute at $8,000 \times g$ to elute the RNA, which was stored at -80°C until further use in downstream applications.

2.9.3 RNA Isolation using TRI Reagent

Cells were trypsinised as per section 2.5.3. Resulting pellets were re-suspended in 1 μL TRI Reagent solution (Thermofisher). The homogenate was incubated for 5 minutes at room temperature. To the homogenate, 100 μL of BCP per 1 mL of TRI Reagent solution was added, mixed well, and incubated at room temperature for 5-15 minutes. The mixture was then centrifuged at $12,000 \times g$ for 10-15 minutes at 4°C , and the aqueous phase was transferred to a fresh tube. Following this, 500 μL of isopropanol per 1 mL of TRI Reagent solution was added, vortexed for 5-10 seconds, and incubated at room temperature for 5-10 minutes. This was followed by centrifugation at $12,000 \times g$ for 8 minutes at $4-25^\circ\text{C}$, after which the supernatant was discarded. To wash the RNA, 1 mL of 75% ethanol per 1 mL of TRI Reagent solution was added, and the mixture was centrifuged at $7,500 \times g$ for 5 minutes. The ethanol was removed, and the RNA pellet was briefly air-dried before being dissolved in the buffer of choice. This method allowed for the efficient extraction, precipitation, and solubilisation of RNA while also isolating DNA and proteins.

2.9.4 DNA Isolation

Genomic DNA was isolated using a high-salt DNA isolation procedure adapted from Laird et al (280). Samples were lysed by adding lysis buffer directly to the tissue or cell pellet, using 0.5 mL for small samples or 5 mL for a 75 cm^2 flask. The lysates were digested at 37°C for cell samples for two to three hours, or at 55°C for tissue samples with agitation, until digestion was complete. The lysis buffer was composed of 100 mM Tris HCl pH 8.5 (5 mL), 0.5 M EDTA (0.5 mL), 10% SDS (1 mL), 5 M NaCl (2 mL), and 20 mg/ml Proteinase K (0.25 mL), with the volume brought up to 50 mL using nuclease free water.

Following lysis, isopropanol precipitation was performed by adding an equal volume of isopropanol to the lysate, and the samples are mixed or swirled until DNA precipitation is complete, approximately 10-20 mins. The DNA was then recovered by lifting the aggregated precipitate from the solution. Excess liquid was dabbed off, and the DNA is dispersed in a pre-labeled Eppendorf tube containing 20 to 500 µl of 10 mM Tris HCl, 0.1 mM EDTA, pH 7.5, depending on the size of the precipitate.

2.9.5 RNA / DNA Quantification

Total RNA and genomic DNA was quantified using a NanoDrop 1000 spectrophotometer (ThermoFisher Scientific). The instrument was first initialised and blanked with 1 µL RNase/DNase-free H₂O. One µL of each sample was loaded individually onto the NanoDrop. The instrument was cleaned between each sample. The NanoDrop returned the nucleic acid concentration in ng/µL and also the 260:280 and 260:230 purity ratios. A 260/280 ratio of ~1.8 is generally accepted as “pure” for DNA; a ratio of ~2.0 is generally accepted as “pure” for RNA.

2.9.6 RNA quality assessment

Each RNA sample was electrophoresed on a 0.8% agarose gel to determine RNA quality. Bands for the 18S and 28S ribosomal subunits were a good indication of RNA integrity. A 0.8% agarose (Sigma-Aldrich) gel was prepared in 100 mL 1X Tris-acetate EDTA (TAE) buffer (Sigma-Aldrich). The agarose was dissolved by heating in a microwave for 2-3 min. Five µL SYBR Safe DNA gel stain (Invitrogen, CA, USA) was added to the solution and the mixture was poured into a gel tray with well forming combs and allowed to set. Once set, the comb was removed, and the gel was covered with 1X TAE buffer. Six µL appropriate ladder (Fisher Scientific) was added to the first well and 15 µL sample was added to subsequent wells. If required, samples were mixed with 2 µL 6X loading dye (Fisher Scientific) before loading on to the gel. Gels were electrophoresed at 120V for 30 min. Gels were visualised using a Licor (CA, USA) gel imaging system.

2.10 PCR

2.10.1 cDNA synthesis for gene expression analysis

cDNA was synthesised using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) by the addition of the following components into a sterile, nuclease-free tube on ice; 1 µg total RNA, 2 µL 10X RT Buffer, 0.8 µL 25X dNTP Mix, 2.0 µL RT Random Primers, 1.0 µL Multiscribe Reverse Transcriptase, 1.0 µL RNase inhibitor and 3.2 µL Nuclease-free H₂O to a final volume of 20 µL. The thermal cycling conditions were as follows; 10 min at 25°C, 120 min at 37°C, 5 min at 85°C. cDNA was stored at -20°C until use.

2.11 circRNA Analysis

2.11.1 Cell line Preparation for circRNA analysis

Cells from each cell line were trypsinized and pelleted following the established protocol. RNA was then extracted using the previously described method. This procedure was performed in triplicate (N=3) for each cell line to ensure robustness. The RNA concentration of each sample was quantified using a NanoDrop, as detailed earlier. Each set of triplicate samples (N=3) was pooled and subsequently divided into two aliquots (n=2) per cell line. These aliquots were sent to Arraystar for circRNA processing and analysis.

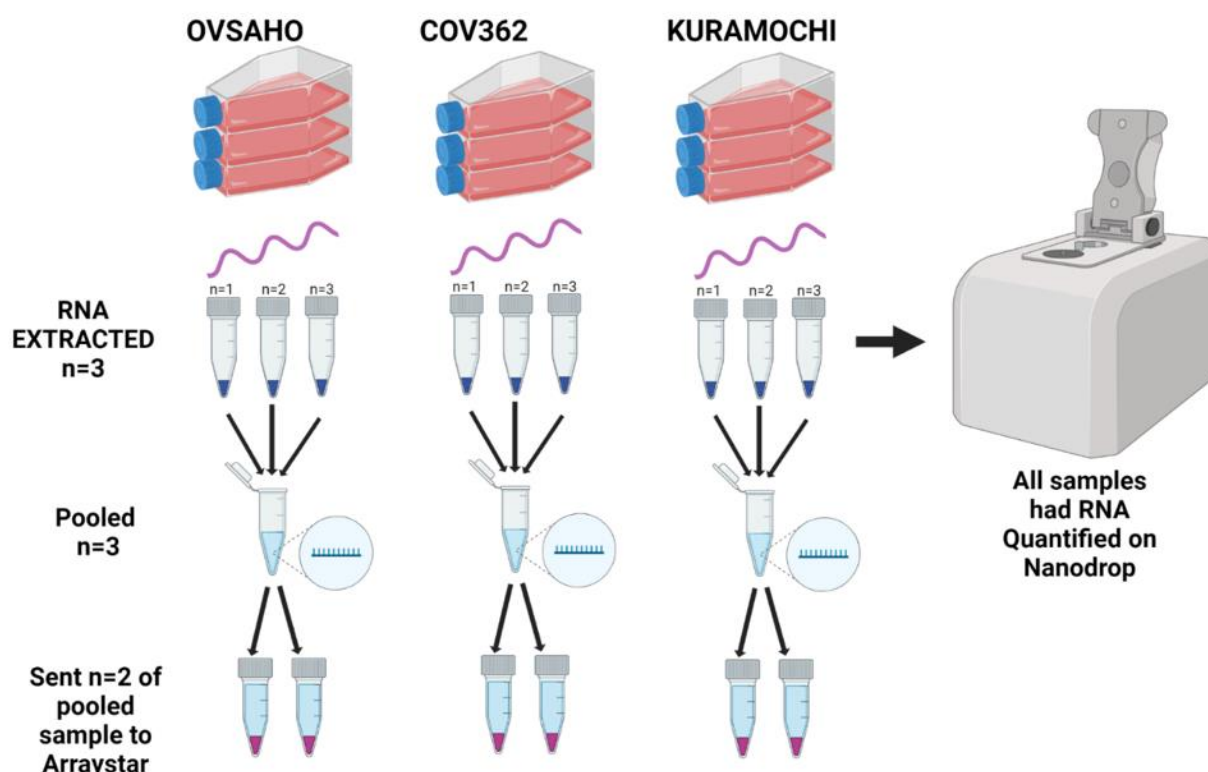


Figure 2-3 Cell Line Preparation and RNA Extraction for circRNA Analysis

2.11.2 circRNA microarray

Sample labelling and array hybridization were performed according to the manufacturer's protocol (Arraystar Inc.). Briefly, total RNAs were digested with Rnase R (Epicentre, Inc.) to remove linear RNAs and enrich circular RNAs. Then, the enriched circular RNAs were amplified and transcribed into fluorescent cRNA utilizing a random priming method (Arraystar Super RNA Labeling Kit; Arraystar). The labeled cRNAs were purified by RNeasy Mini Kit (Qiagen). The concentration and specific activity of the labeled cRNAs (pmol Cy3/ μ g cRNA) were measured by NanoDrop ND-1000. 1 μ g of each labeled cRNA was fragmented by adding 5 μ l 10 $\times$ Blocking Agent and 1 μ l of 25 $\times$ Fragmentation Buffer, then heated the mixture at 60 $^{\circ}$ C for 30 min, finally 25 μ l 2 $\times$ Hybridization buffer was added

to dilute the labeled cRNA. 50 µl of hybridization solution was dispensed into the gasket slide and assembled to the circRNA expression microarray slide. The slides were incubated for 17 hours at 65°C in an Agilent Hybridization Oven. The hybridized arrays were washed, fixed and scanned using the Agilent Scanner G2505C.

2.11.3 circRNA validation

2.11.3.1 Primer design for circRNA

Validation of circRNAs can be achieved using qPCR-based assays designed using outward facing primers as illustrated in **Figure 2-4**. These are outlined in **Appendix 1**.

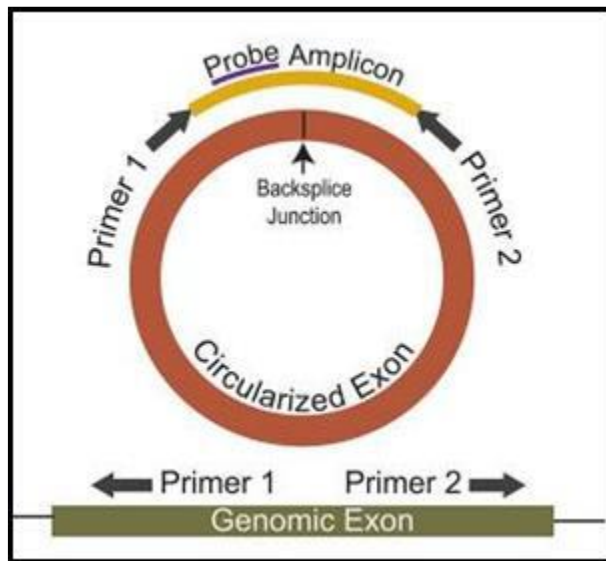


Figure 2-4 Primer design for circRNA validation.

Image taken from Jeck et al. (66).

2.11.3.2 Preparation of histological patient samples

Histological formalin-fixed paraffin-embedded (FFPE) tumour blocks were obtained from the institutional biobank. Tissue cores were excised from representative tumour regions and mechanically disrupted using a sterile pestle and mortar. The homogenised tissue was immediately transferred to TRIzol™ reagent for RNA extraction, according to the manufacturer's protocol. All procedures were performed in compliance with institutional ethical approvals and biobank material transfer agreements.

2.11.3.3 PCR Analysis and Fold Change Calculation

qPCR was performed as previously described, and data were analysed using the comparative Ct ($\Delta\Delta Ct$) method. For each sample, the Ct value of the target circRNA was normalised to the mean Ct of a housekeeping gene to obtain the ΔCt . $\Delta\Delta Ct$ values were calculated by subtracting the ΔCt of the reference sample from that of each experimental sample. Fold change (FC) was derived as $2^{-\Delta\Delta Ct}$. Each sample was run in triplicate, and the mean and standard deviation (SD) of $\Delta\Delta Ct$ were calculated across technical replicates. SD in fold change space was obtained using the delta method ($SD_{FC} =$

$FC_{\text{mean}} \times \ln(2) \times SD_{\Delta\Delta Ct}$) and 95% confidence intervals were generated by calculating the limits in $\Delta\Delta Ct$ space and back-transforming. All analyses were performed in Prism version 10.3.1 and Microsoft Excel.

2.11.4 circRNA Data Analysis

2.11.4.1 Bioinformatics

Arraystar™ provided bioinformatic data to accompany circRNA array data in the form of venn diagrams, volcano plots and heat maps. A further collaboration with a bioinformatic group in NUIG has been established for assistance with the biomedical and genomics data results. Analysis of circRNA microarray data will include sample quality control, exploratory data analysis (PCA plots/sample correlation heatmaps), identification of differentially expressed circRNAs using linear modelling approaches and visualization of result using e.g. volcano plots/heatmaps.

2.11.4.2 Filtering of Differentially Expressed circRNAs

When comparing two groups of profile differences (such as disease vs. control), the “fold change” (i.e. the ratio of the group averages) between the groups for each circRNA was computed. The statistical significance of the difference may be conveniently estimated by t-test. circRNAs having fold changes ≥ 2 and p-values > 0.05 are selected as the significantly differentially expressed. One can filter the analysis outputs and rank the differentially expressed circRNAs according to fold change, p-value etc., and using Microsoft Excel’s Data/Sort & Filter functionalities.

2.11.4.3 Annotation For circRNA/miRNA Interaction

The circRNA/miRNA interaction was predicted with Arraystar's home-made miRNA target prediction software based on TargetScan(281) & miRanda(282), and the differentially expressed circRNAs within all the comparisons were annotated in detail with the circRNA/miRNA interaction information.

2.11.5 Functional Enrichment Analysis

Functional enrichment analysis was used to explore the biological relevance of differentially expressed genes. GO analysis was performed using the topGO package in Bioconductor to identify enriched biological processes, cellular components, and molecular functions. Fisher’s exact test was used to assess significance, with a p-value threshold of 0.05. Results included enriched GO terms, gene counts, fold enrichment, adjusted p-values, and enrichment scores. Unsupervised clustering was adopted whereby grouping samples based solely on circRNA expression similarity, without providing prior information about their treatment status. Visual outputs included bar plots, dot plots, pie charts, and GO directed acyclic graphs. KEGG pathway analysis was carried out to identify pathways associated with the differentially expressed genes. Significance was calculated using EASE, Fisher, or Hypergeometric tests. Outputs included pathway names, enrichment scores, gene ratios, false discovery rates, and lists of involved genes. Pathways were visualised using bar plots, dot plots, and KEGG maps showing upregulated and downregulated genes.

Demographic and Epidemiologic Landscape of Ovarian Cancer in Ireland

Chapter Three

3 Demographic and Epidemiologic Landscape of OC in Ireland

3.1 Introduction

3.1.1 Genetic Landscape of OC in Ireland

Harbouring a germline *BRCA1* or *BRCA2* (*gBRCA1/2*) pathogenic variant (PV), now more commonly referred to as a clinically actionable variant (CAV) is a major risk factor for developing HGSOC, with an estimated lifetime risk of 40–50% and 10–20%, respectively (98, 283), compared to 1.5% in the general population (284). *BRCA1/2* mutated OC is associated with a better OS (7), a finding that seems to be attributable to an increased sensitivity to DNA damaging agents, particularly platinum compounds (285). Furthermore, *BRCA1/2* PVs are predictive of response to PARPi and can therefore help select the best candidates for treatment (13, 14). Owing to these advances, *BRCA1/2* germline and somatic sequencing is recommended by European and US guidelines for patients diagnosed with HGSOC, HGEOC, Figure 2-2 and FTC, even in the absence of a family history (52, 286). In 2019 in Ireland, a feasibility study, the T-BRCA study (CTRIAL-IE 18-0) (88), aimed to evaluate the feasibility of routine upfront systematic OC tumour tissue testing for *BRCA1/2* (*tBRCA1/2*), regardless of their personal or family history of breast/OC. The results of this study enabled the introduction of a publicly funded direct order testing for *BRCA* mutations by the National Cancer Control Program (NCCP) for Consultant Medical Oncologists to allow timely access to testing to help inform treatment decisions with PARPi in 2020 (287).

These developments underscore the growing importance of understanding genetic factors in OC, particularly as the prevalence of germline *BRCA1/2* PVs among OC patients can vary significantly across different population(s) and can be influenced by various factors such as ethnicity, geographic location, and familial predisposition (288). Despite extensive research on the prevalence of *BRCA1/2* PVs in OC cohorts worldwide (289-294), there is a notable lack of nationwide published data in the Irish population, with existing studies limited to very small cohorts(295). Therefore, understanding and characterizing the prevalence of *BRCA1/2* PVs in a large nationwide Irish OC cohort spanning all factors mentioned above is essential for informing targeted treatment strategies, genetic counselling practices, and the development of effective screening programs.

3.1.2 Characteristics and Challenges of OC in Young Women

Although OC is typically associated with postmenopausal women, it also affects a smaller but significant group of younger individuals—defined here as those aged 50 years or younger (296). While OC is relatively rare in this age group (297), its impact is disproportionately profound. These patients often face complex challenges, including the possibility of hereditary cancer syndromes (283), delayed diagnosis due to lower clinical suspicion (298), and distinct psychosocial stressors (50). A diagnosis during early adulthood or adolescence frequently coincides with critical life stages,

raising urgent issues around fertility preservation, long-term health planning, and career or family responsibilities (299).

In addition to these clinical and psychosocial considerations, the biological landscape of OC in young people differs substantially from that seen in older women. As outlined in Section 1.1.5, OC is a heterogeneous disease composed of multiple histological subtypes, each with distinct molecular, genetic, and clinical characteristics. As demonstrated in **Table 1-1** the distribution of these subtypes is age dependent. HGSOC, the most common form overall, predominates in postmenopausal women. In contrast, younger patients are more likely to present with rarer epithelial subtypes—such as LGSOC, MOC, and ENOC, as well as non-epithelial tumours, including germ cell and sex cord-stromal tumours. These subtypes often carry different genetic drivers, display unique tumour biology, and respond differently to treatment.

Taken together, these factors highlight that OC in young individuals is not simply a younger manifestation of the same disease, but a distinct clinical and biological entity. This distinction reinforces the importance of dedicated research into YOC with a focus on age-specific diagnostic strategies, therapeutic approaches, genetic counselling, and supportive care models.

3.2 Aims and Objectives

3.2.1 General Aims

The aim of this chapter was to assess the demographic and epidemiologic landscape of OC in Ireland through a well-defined large cohort of patients

3.2.2 Hypotheses

- The genetic landscape of OC in Ireland is unique, with a distinctive prevalence of *BRCA1* and *BRCA2* PVs
- Young Irish people diagnosed with OC under age 50 years exhibit distinct clinical and genetic characteristics.

3.2.3 Objectives

The objectives of this chapter are:

- To report the prevalence of germline and somatic *BRCA1/2* PVs in an Irish OC cohort
- To characterize clinical and genetic features associated with germline and somatic *BRCA1/2* CAVs in HGSOC
- To evaluate the cost-effectiveness and diagnostic efficiency of integrated vs. reflex *BRCA* testing strategies in OC
- To report the clinico-pathological characteristics of young Irish people diagnosed with OC under age 50 years

3.3 Results

3.3.1 Nationwide Prevalence and Clinical Impact of *BRCA1/2* Mutations in OC

3.3.2 Patient Cohort Characteristics

A total of 535 individuals with ovarian cancer were referred for centralised *BRCA* testing (**Table 3-1**).

The median age at time of referral was 65.5 years (range 27.2yrs – 88.6yrs). Of these 535 patients, 462 (86.4%) had a diagnosis of HGSOC and 48 (9.0%) had a diagnosis of HGEOC. Twelve patients (2.2%) had no testing results available, either due to issues with sample quality or samples not received. Patients were referred from 31 referral centres spanning throughout the Republic of Ireland to two large tertiary referral centres which act as centralised national testing centres; 71 patients (13.3%) were referred to Beaumont Hospital and 464 patients (86.7%) were referred to St James Hospital.

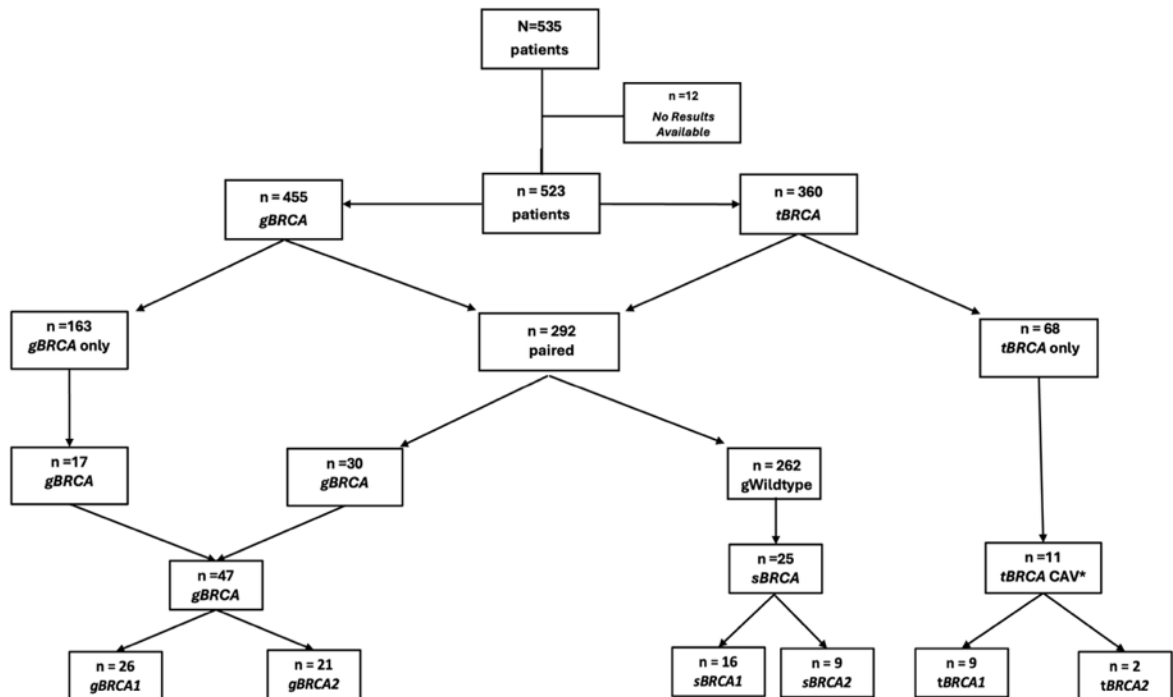
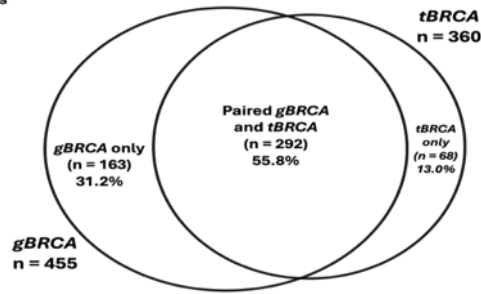
Patient Characteristics		Patient (n, %)
Subtype	HGSOC	462 (86.4%)
	HGEOC	48 (9.0%)
	Unspecified	25 (4.6%)
Sex	Female	525 (98.1%)
	Male	1 (<1%)
	Unspecified	9 (1.6%)
		Years (yrs)
Age	Median	65.5 yrs
	Min	27.2 yrs
	25%	57.0 yrs
	50%	65.4 yrs
	75%	72.4 yrs
	Max	88.6 yrs

Table 3-1 Characteristics of Cohort Referred for Testing

3.3.3 Prevalence of Germline BRCA1/2 Mutations

Of the 535 patients included in the cohort, 455 (85.0%) underwent *gBRCA* testing, 360 (67.2%) underwent somatic *BRCA* (*sBRCA*) testing, and 292 (54.6%) had paired germline and somatic testing. Among those with negative *gBRCA* results who underwent paired testing, *sBRCA* CAVs were identified and used to define the *sBRCA* rate. Eight patients underwent *tBRCA* testing alone (**Figure 3-1, Table 3-2**). Among the 455/535 underwent *gBRCA*, of which 47 (10.3%) were found to have germline PV, including 26 (5.7%) in *BRCA1* and 21 (4.6%) in *BRCA2*. Additionally, 7 patients (1.5%) had a variant of uncertain significance (VUS). Of the 360 (67.2%) underwent somatic testing, 292 (54.6% of total cohort) also had a paired germline test. Within this group, 30 patients (10.3%) were identified with a *gBRCA* PV, all of whom had a matching *sBRCA* PV. For the 292 patients who underwent paired testing and had a negative *gBRCA* result (n=262), 25 (9.5%) were identified with a *sBRCA* PV, comprising 16 (6.1%) in *BRCA1* and 9 (3.4%) in *BRCA2*. Among the 68 patients who underwent *tBRCA* testing in isolation, 11 (16.1%) were identified with a *BRCA* PV, including 9 (13.2%) and 2 (2.9%) in *BRCA2* and *BRCA1* respectively. Among HGSOc patients, paired germline and somatic testing was performed in 253/462 (54.8%); *sBRCA* CAVs were identified in 23/224 (10.3%) patients with a negative germline result, more commonly in *BRCA1* (6.7%)

N = 523
patients



gBRCA = Germline BRCA testing, sBRCA = Somatic BRCA testing, tBRCA = Tumour BRCA testing.

*Tumour BRCA testing along, therefore CAV (Clinically Actionable Variant) aetiology unknown

Figure 3-1 BRCA Testing Outcomes: Venn and CONSORT Diagrams

The Venn diagram shows overlap between germline (gBRCA), tumour (tBRCA), and somatic (sBRCA) testing, with sBRCA results derived from somatic testing in patients negative for gBRCA. The CONSORT diagram summarises overall testing outcomes across all three modalities.

Germline and Somatic <i>BRCA1/2</i> results across entire population		Patient (n, %)	<i>BRCA1/2</i> CAV Identified n, (%)	<i>BRCA1</i> PV n, (%)	<i>BRCA2</i> PV n, (%)	<i>BRCA1/2</i> VUS Identified n, (%)	<i>BRCA1</i> VUS n, (%)	<i>BRCA2</i> VUS n, (%)
	Total Patients Referred for Testing	535 (100%)						
	No Testing results available	12 (2.2%)						
	<i>BRCA</i> Result Available	523 (97.8%)						
	Total <i>gBRCA1/2</i>	455 (85.1%)	47 (10.3%)	26 (5.7%)	21 (4.6%)	7 (0.02%)	3 (0.01%)	4 (0.01%)
	Total <i>sBRCA1/2</i>	360 (67.3%)	36 (10.0%)	25 (6.9%)	11 (3.0%)	3 (0.8%)	2 (0.6%)	1 (0.3%)
	Paired Germline and Somatic Testing	292 (54.6%)						
	<i>gBRCA1/2</i>	292 (54.6%)	30 (10.3%)	16 (5.5%)	14 (4.8%)	6 (2.0%)	3 (1.0%)	3 (1.0%)
	<i>sBRCA1/2</i> with a negative germline test	262 (49.0%)	25 (9.5%)	16 (6.1%)	9 (3.4%)	3 (0.01%)	2 (<0.01%)	1 (<0.01%)
	Somatic testing in isolation	68 (12.7%)						
	t <i>BRCA1/2</i>	68 (12.7%)	11 (16.1%)	9 (13.2%)	2 (2.9%)			

Table 3-2 Germline and Somatic *BRCA1/2* CAV prevalence.

Germline and Somatic <i>BRCA1/2</i> results in HGSOC patients only	Total Patients Referred for Testing	462 (100%)						
	No Testing results available	10 (2.2%)						
	<i>BRCA</i> Result Available	452 (97.8%)						
	Total <i>gBRCA1/2</i>	391 (84.6%)	45 (11.5%)	25 (6.4%)	20 (5.1%)	7 (1.8%)	3 (0.8%)	4 (1.0%)
	Total <i>gBRCA1/2</i>	315 (68.2%)	33 (10.5%)	23 (7.3%)	10 (3.2%)	1 (0.3%)	0 (0.0%)	1 (0.3%)
	Paired Germline and Somatic Testing	253 (54.8%)						
	<i>gBRCA1/2</i>	253 (54.8%)	29 (11.4%)	15 (5.9%)	14 (5.5%)	6 (2.4%)	3 (1.2%)	3 (1.2%)
	<i>sBRCA1/2</i> with a negative germline test	224 (54.8%)	23 (10.3%)	15 (6.7%)	8 (3.6%)	1 (0.4%)	1 (0.4%)	0 (0.0%)

Table 3-3 Germline and Somatic *BRCA1/2* CAV prevalence for HGSOC subgroup

3.3.4 Characteristics of the BRCA1/2 Population

Patients with *gBRCA1/2* CAV tended to be significantly younger than patients with *gBRCA1/2* wild-type (median age 58.0 vs. 66.5 years, $U = 4818$, $p = <0.01$) at time of referral (**Figure 3-2**). Similarly, patients with *sBRCA1/2* CAV OC were younger than patients with *sBRCA1/2* wild-type (median age 57.9 vs. 64.0 years, $U = 2576.0$, $p = 0.07$) at time of referral. The comparison of median ages between patients with germline and somatic *BRCA1/2* PVs revealed no statistically significant difference ($p = 0.576$), indicating comparable age distributions within these groups.

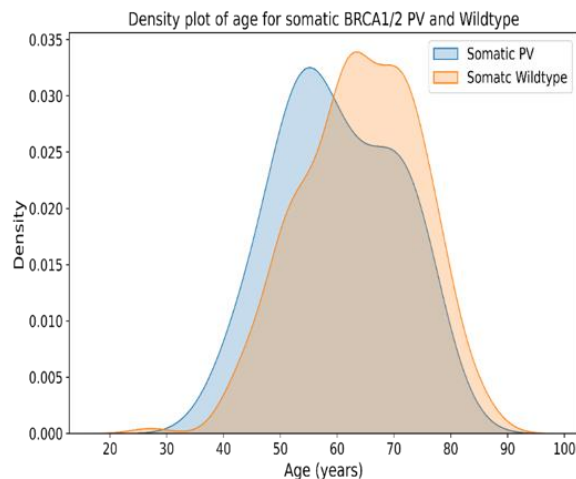
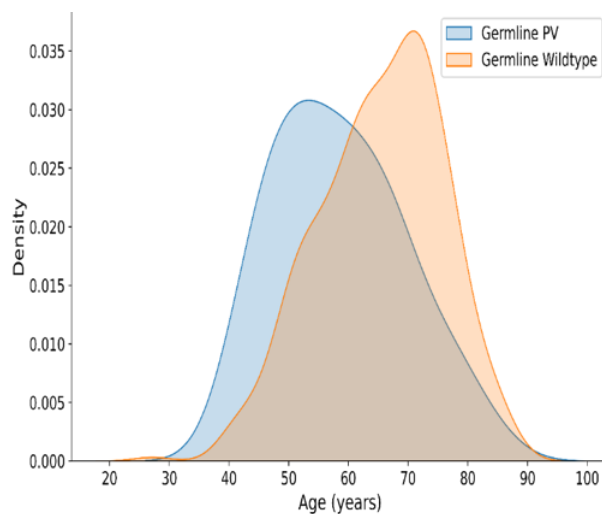


Figure 3-2 Density plots of age distribution in Germline/Somatic BRCA1/2 vs. wildtype

Age at diagnosis was treated as a numerical variable and is shown as density distributions for *BRCA1/2*-mutated and wildtype groups. Comparisons between groups were performed using the Mann–Whitney U test.

3.3.5 Characteristics of *BRCA1/2* Variants

Six separate *gBRCA1/2* PVs were discovered in more than one patient. The most common of these was *BRCA1* CAV *c.5266dupC*, which was noted in six patients (1.25%) (**Table 3-4**). This CAV was documented across various regions in Ireland, including eastern, midland, and southern parts of the country/ The *BRCA1* deletion *c.1175_1214del* was found in 4 patients, all originating from referral facilities in the south of Ireland. The deletion *c.4398_4402del* was the most common *BRCA2* PV, identified in 4 patients, all originating from referral facilities in the east of Ireland (**Figure 3-3**). There were an additional 27 *gBRCA* variants identified in single cases in the study cohort, totalling 33 unique *gBRCA* variants (**Table 3-5**). Among the 262 patients who underwent *sBRCA* tumour testing with a negative germline test, there were no recurring variants, resulting in 16 unique variants in *BRCA1* and 10 unique variants in *BRCA2*. There were also four large genomic rearrangements (LGA) identified within the 33 unique *gBRCA1 PV's* - '*BRCA1:del_exon_3*', '*BRCA1_del_exons_1-22*', '*BRCA1_del_exons_13-19*' and '*BRCA2_del_exons_19-20*'. The distribution of identified germline and somatic *BRCA1/2* PVs across the protein-coding regions is illustrated in **Table 3-4**, highlighting the localization of mutations along the *BRCA1* and *BRCA2* proteins.

	Gene	N (%)	HGVS* cDNA	HGVS* Protein	Molecular consequence
Germline	BRCA1	6 (1.25%)	c.5266dupC	p.Gln1756Profs*74	Frameshift
Germline	BRCA1	4 (0.83%)	c.1175_1214del	p.Leu392Glnfs*5	Frameshift
Germline	BRCA2	4 (0.83%)	c.4398_4402del	p.Leu1466Phefs*2	Frameshift
Germline	BRCA1	3 (0.62%)	c.181T>G	p.Cys61Gly	Missense
Germline	BRCA2	3 (0.62%)	c.4638del	p.Phe1546fs*22	Frameshift
Germline	BRCA2	2 (0.41%)	c.6275_6276del	p.Leu2092Profs*7	Frameshift

Table 3-4 Most frequently reported *gBRCA1/2* CAV



Figure 3-3 Geographical distribution of most common *BRCA* PV's in Ireland

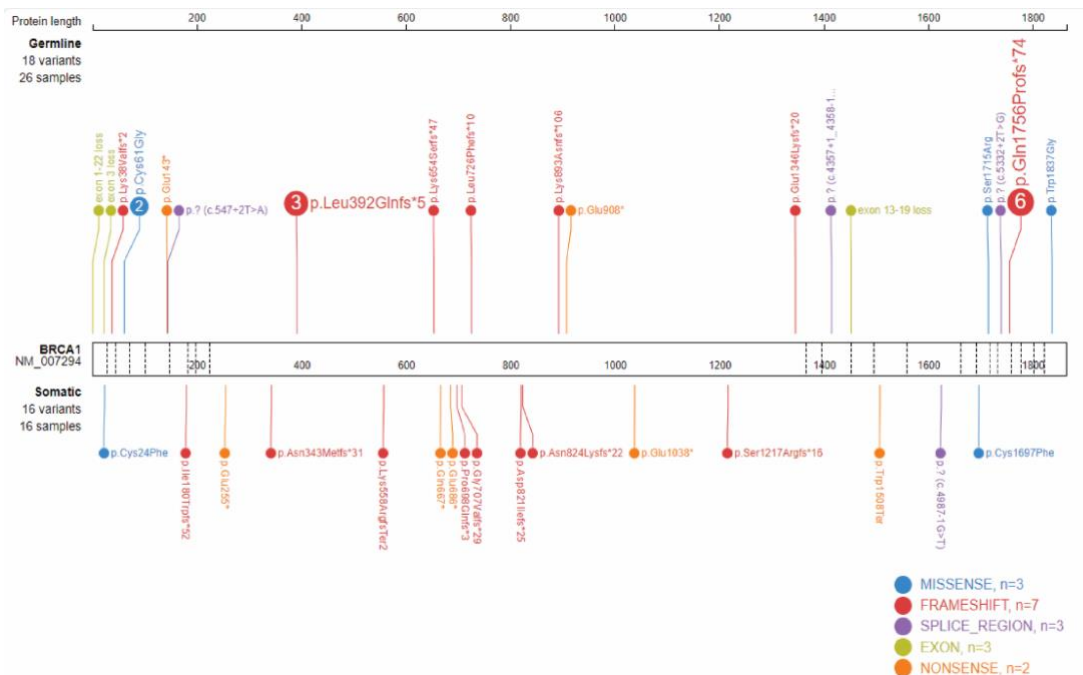
Study ID	Age	Hugo Symbol	Protein Change	Mutation_Type	HGVS_cDNA	Exon
1	47	<i>BRCA1</i>	<i>p.Lys38Valfs*2</i>	Frameshift Variant	c.112_113del	-
2	44	<i>BRCA1</i>	<i>p.Leu392Glnfs*5</i>	Frameshift Variant	c.1175_1214del	9
3	52	<i>BRCA1</i>	<i>p.Leu392Glnfs*5</i>	Frameshift Variant	c.1175_1214del	9
4	76	<i>BRCA1</i>	<i>p.Leu392Glnfs*5</i>	Frameshift Variant	c.1175_1214del	9
5	58	<i>BRCA2</i>	<i>p.Val464G1yfs*3</i>	Frameshift Variant	c.1389_1390del	10
6	44	<i>BRCA1</i>	<i>p.Lys654Serfs*47</i>	Frameshift Variant	c.1961delA	9
7	68	<i>BRCA1</i>	<i>p.Leu726Phefs*10</i>	Frameshift Variant	c.2176del	9
8	65	<i>BRCA1</i>	<i>p.Lys893Asnfs*106</i>	Frameshift Variant	c.2679_2682del	9
9	62	<i>BRCA2</i>	<i>p.Lys1239Asnfs*20</i>	Frameshift Variant	c.3717del	11
10	46	<i>BRCA1</i>	<i>p.Glu1346Lysfs*20</i>	Frameshift Variant	c.4035del	9
11	58	<i>BRCA2</i>	<i>p.Thr1426Asnfs*12</i>	Frameshift Variant	c.4276dup	11
12	69	<i>BRCA2</i>	<i>p.Leu1466Phefs*2</i>	Frameshift Variant	c.4398_4402del	11
13	65	<i>BRCA2</i>	<i>p.Leu1466Phefs*2</i>	Frameshift Variant	c.4398_4402del	11
14	60	<i>BRCA2</i>	<i>p.Leu1466Phefs*2</i>	Frameshift Variant	c.4398_4402del	11
15	53	<i>BRCA2</i>	<i>p.Leu1466Phefs*2</i>	Frameshift Variant	c.4398_4402del	11
16	71	<i>BRCA2</i>	<i>p.Glu1493Valfs*10</i>	Frameshift Variant	c.4478_4481del	11
17	58	<i>BRCA2</i>	<i>p.Phe1546LeufsTer22</i>	Frameshift Variant	c.4638del	11
18	68	<i>BRCA2</i>	<i>p.Phe1546Leufs*22</i>	Frameshift Variant	c.4638del	11
19	69	<i>BRCA2</i>	<i>p.Phe1546Leufs*22</i>	Frameshift Variant	c.4638del	11
20	50	<i>BRCA1</i>	<i>p.Gln1756Profs*74</i>	Frameshift Variant	c.5266dup	18
21	84	<i>BRCA1</i>	<i>p.Gln1756Profs*74</i>	Frameshift Variant	c.5266dupC	18
22	61	<i>BRCA1</i>	<i>p.Gln1756Profs*74</i>	Frameshift Variant	c.5266dupC	18
23	50	<i>BRCA1</i>	<i>p.Gln1756Profs*74</i>	Frameshift Variant	c.5266dupC	18
24	41	<i>BRCA1</i>	<i>p.Gln1756Profs*74</i>	Frameshift Variant	c.5266dupC	18

25	63	<i>BRCA1</i>	<i>p.Gln1756Profs*74</i>	Frameshift Variant	c.5266dupC	18
26	53	<i>BRCA2</i>	<i>p.Leu2092Profs*7</i>	Frameshift Variant	c.6275_6276del	11
27	61	<i>BRCA2</i>	<i>p.Leu2092Profs*7</i>	Frameshift Variant	c.6275_6276del	11
28	79	<i>BRCA2</i>	<i>p.Lys2162Asnfs*5</i>	Frameshift Variant	c.6486_6489del	11
29	78	<i>BRCA2</i>	<i>p.Lys2538Asnfs*13</i>	Frameshift Variant	c.7614delA	15
30	50	<i>BRCA2</i>	<i>p.Ile2588Phefs*60</i>	Frameshift Variant	c.7762_7764delinsTT	16
31	53	<i>BRCA1</i>		Large Genomic Rearrangement	BRCA1:del_exon_3	3
32	53	<i>BRCA1</i>		Large Genomic Rearrangement	BRCA1_del_exons_1-22	1_22
33	63	<i>BRCA1</i>		Large Genomic Rearrangement	BRCA1_del_exons_13-19	13_19
34	77	<i>BRCA2</i>		Large Genomic Rearrangement	BRCA2_del_exons_19-20	19-20
35	51	<i>BRCA1</i>	<i>p.Cys61Gly</i>	Missense Variant	c.181T>G	3
36	46	<i>BRCA1</i>	<i>p.Cys61Gly</i>	Missense Variant	c.181T>G	3
37	49	<i>BRCA1</i>	<i>p.Ser1715Arg</i>	Missense Variant	c.5145C>G	16
38	41	<i>BRCA1</i>	<i>p.Trp1837Gly</i>	Missense Variant	c.5509T>G	22
39	57	<i>BRCA2</i>		Splice Acceptor Variant	c.8331+1_8332-1_8632+1_8633-1del	-
40	53	<i>BRCA1</i>		Splice Donor Variant	c.5332+2T>G	-
41	45	<i>BRCA1</i>		Splice Donor Variant	c.547+2T>A	-
42	58	<i>BRCA1</i>		Splice Donor Variant, Splice Donor 5th Base Variant, Intron Variant	c.4357+1_4358-1_4986+1_4987-1del	-
43	57	<i>BRCA2</i>	<i>p.Gln486*</i>	Stop Gained	c.1456C>T	10
44	72	<i>BRCA2</i>	<i>p.Glu790*</i>	Stop Gained	c.2368G>T	11

45	67	<i>BRCA1</i>	<i>p.Glu908*</i>	Stop Gained	c.2722G>T	9
46	46	<i>BRCA1</i>	<i>p.Glu143*</i>	Stop Gained	c.427G>T	5
47	64	<i>BRCA2</i>	<i>p.Tyr1655*</i>	Stop Gained	c.4965C>A	11

Table 3-5 PVs identified in *BRCA1/2*: mutation type, and genomic details.

A



B

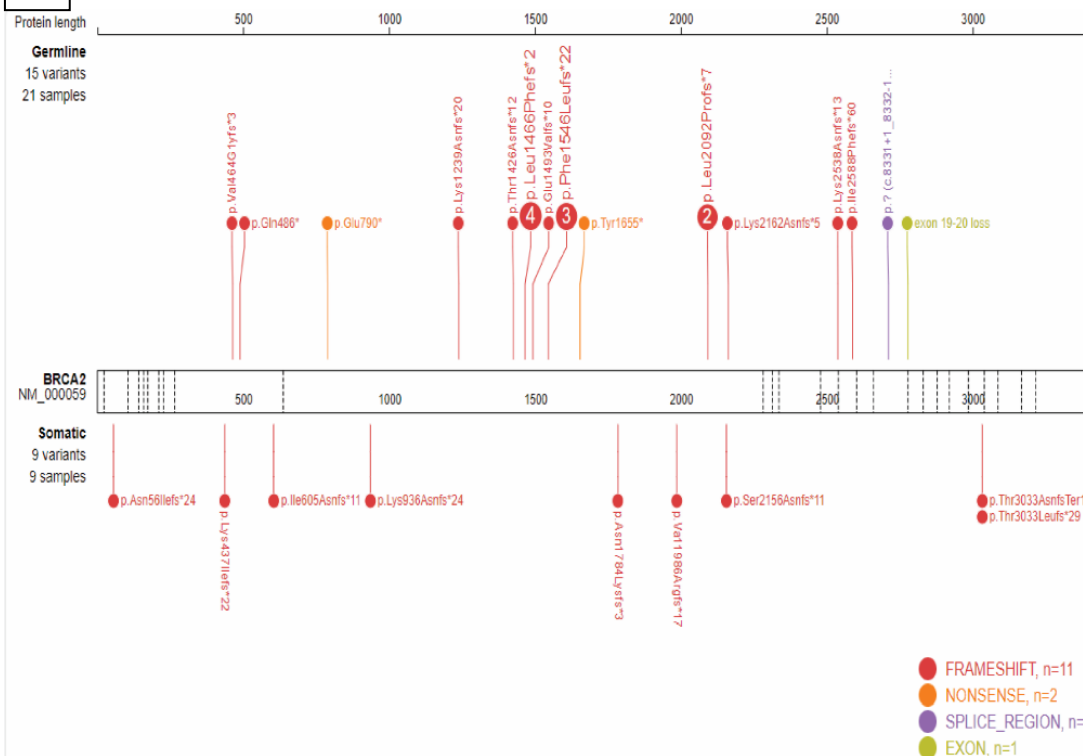


Figure 3-4 BRCA1/2 Mutation Mapping with Lollipop Plots

(A). Lollipop plot of the localization of the identified germline and somatic *BRCA1* mutations in *BRCA1* proteins. (B). Lollipop plot of the localization of the identified germline and somatic *BRCA2* mutations in *BRCA2* proteins. Numbers on the protein graph correspond to amino acid locations; dashed lines delineate exons. Figure created using ProteinPaint software (300).

3.3.6 Economic Evaluation of BRCA Testing in Ovarian Cancer

This study also modelled the cost-effectiveness and turnaround time of three *BRCA* testing strategies for OC patients at St. James's and Beaumont Hospitals: integrated testing, tumour-first reflex testing, and germline-first reflex testing. Using retrospective data from 292 patients who underwent paired testing, the model showed that integrated testing - where both germline and tumour *BRCA* testing are performed simultaneously - identified all *BRCA* PVs, including 30 *gBRCA* PVs (10.3%) and an additional 25 *sBRCA* PVs (9.5%) among germline-negative patients (**Table 3-2**). This approach had the highest overall cost at €408,800 (**Table 3-6**). In comparison, reflex testing models—where testing begins with either the tumour or germline sample, and the second test is triggered by the result—were more cost-effective, costing €219,200 (tumour-first) and €251,600 (germline-first), representing cost reductions of 46.4% and 38%, respectively. However, reflex approaches carried the risk of missing 8.5% of somatic cases and two LGRs. Integrated testing also had a longer median turnaround time (35 days) compared to reflex strategies (26 days).

Testing Strategy	Step	Cost (€) per Test	Tests (n)	Subtotal (€)	<i>BRCA</i> Identified (n, %)	<i>gBRCA</i> Missed (%)	<i>sBRCA</i> Missed (%)	Total Cost (€)
Integrated Testing	1. Germline Testing	800	292	233,600	30/292 (10.3%)	0%	0%	408,800
	1. Tumour Testing	600	292	175,200	30/292 (10.3%)* + 25 <i>sBRCA</i>	0%	0%	
Reflex: <i>tBRCA</i> → <i>gBRCA</i>	Tumour Testing	600	292	175,200	55/292 (18.8%)**	0%	0%	219,200
	2. Germline (if <i>tBRCA</i> +))	800	55	44,000	30/55 (10.3%)*	<1% (2 LGR)**	0%	
Reflex: <i>gBRCA</i> → <i>tBRCA</i>	Germline Testing	800	292	233,600	30/292 (10.3%)	0%	8.5%	251,600
	Tumour (if <i>gBRCA</i> -)	600	30	18,000	25/262 <i>sBRCA</i>	0%	8.5%	

Table 3-6 Cost Analysis and Detection Sensitivity of Integrated and Reflex Testing Models

*Paired testing identified 30 *gBRCA* PVs (10.3%), all identified within the tumour

**LGR's may have been missed due to the inherent complexity of detecting these variants in tumour samples

3.3.7 Clinico-pathological and genetic characteristics of Young People with OC

3.3.8 Young People with OC Study Patient Cohort

Over the period from March 2005 to September 2023, a total of 950 patients with malignant disease consented to the Gynaecological Disease Research Biobank. Among these, 140 patients (14.7%) were identified as having invasive ovarian cancer diagnosed under the age of 50. The median age at diagnosis within this subgroup was 42 years, with an age range of 16 to 50 years.

3.3.9 Clinicopathological Features

The most common histologic subtype was HGSOC, which accounted for 43.6% of cases, followed by MOC (17.9%) and ENOC (11.4%) cancers (**Table 3-7**). Most patients were diagnosed at stages 1 (45.0%) or 3 (37.9%), with a smaller proportion at stages 2 (5.0%) and 4 (10.0%). A significant portion of tumours were high grade (58.6%), with 30.0% classified as low grade. Linear regression analysis demonstrated a statistically significant inverse association between age at diagnosis and histologic subtype in YOC (Pearson correlation coefficient = -0.29 , $p = 0.001$) (**Figure 3-5**). The regression equation ($Subtype = 7.38 - 0.09 \times Age\ at\ Diagnosis$) indicates that younger patients were more likely to present with subtypes typically associated with earlier onset, such as germ cell and stromal tumours.

Characteristic	n (%)
Histologic Subtype	
All Patients	140 (100%)
HGSOC	61 (43.6%)
Mucinous	25 (17.9%)
Endometrioid	16 (11.4%)
Clear Cell	10 (7.1%)
LGSOC	9 (6.4%)
Germ Cell	8 (5.7%)
Stromal	7 (5.0%)
Mixed	3 (2.1%)
Sarcoma	1 (0.7%)
Stage at Diagnosis	
Stage I	63 (45.0%)
Stage III	53 (37.9%)
Stage IV	14 (10.0%)
Stage II	7 (5.0%)
Unknown	3 (2.1%)
Tumour Grade	
High Grade (G3)	82 (58.6%)
Low Grade (G1 & G2)	42 (30.0%)
Unknown	16 (11.4%)

Table 3-7 Clinicopathological Characteristics of the Young-Onset OC Cohort

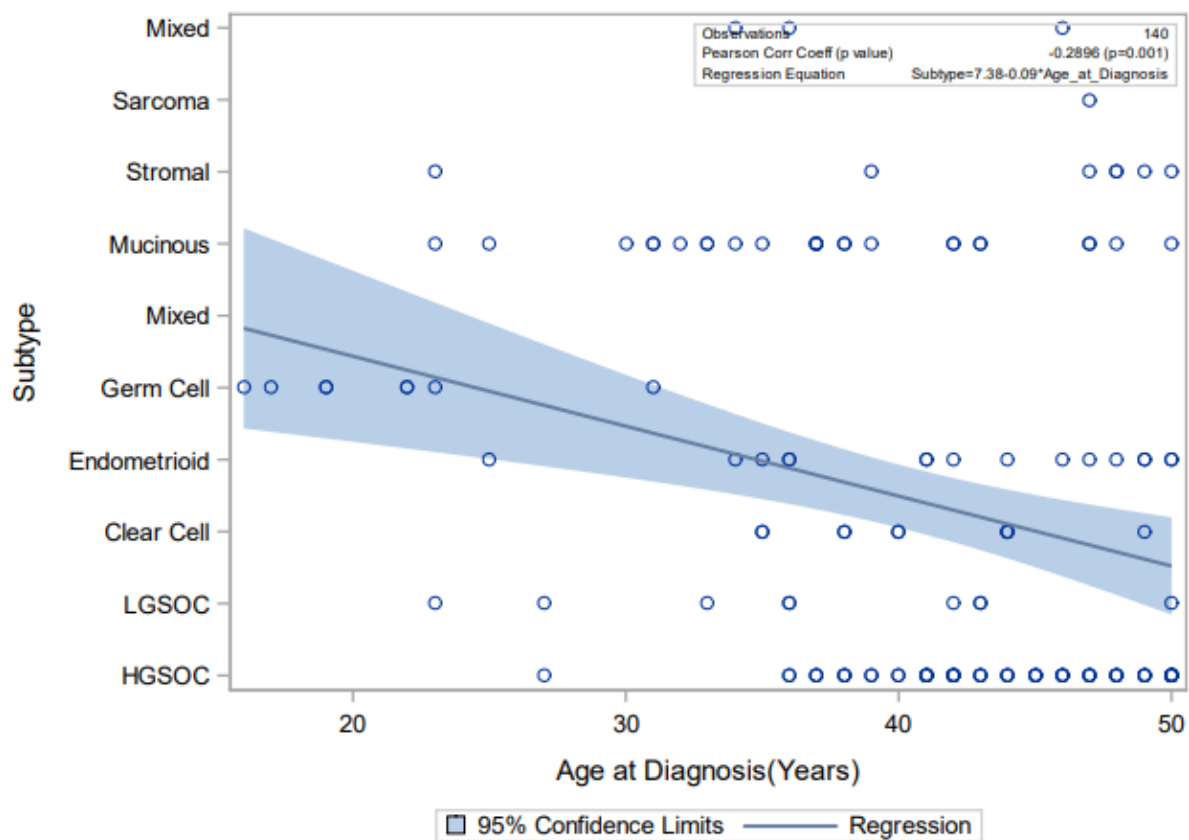


Figure 3-5 Regression Analysis of Age at Diagnosis by Histologic Subtype in Young-Onset OC

3.3.10 Survival Outcomes for Young People with OC

The survival analysis across all patients (n=140) revealed a median OS of 10.2 years, with a five-year OS rate of 60.1%. The survival rate at 15 years was 43.3% (**Figure 3-6**). When stratified by histological subtype, HGSOc had the lowest survival rates, with a five-year OS of 42.0% and a 10-year OS of 28.9%. In contrast, patients with germ cell tumours showed a much higher 5-year OS of 76.0% (**Table 3-8**). The survival rates also varied by stage at diagnosis across the entire young-onset cohort, with stage I patients demonstrating the highest 5-year OS rate of 93.0%, while those diagnosed at stage III and IV had considerably lower OS rates of 33.1% and 34.8%, respectively. (**Table 3-9**). Low-grade tumours had better survival (5-year OS 84.5%) compared to high-grade tumours (44.9%) (**Table 3-10**).

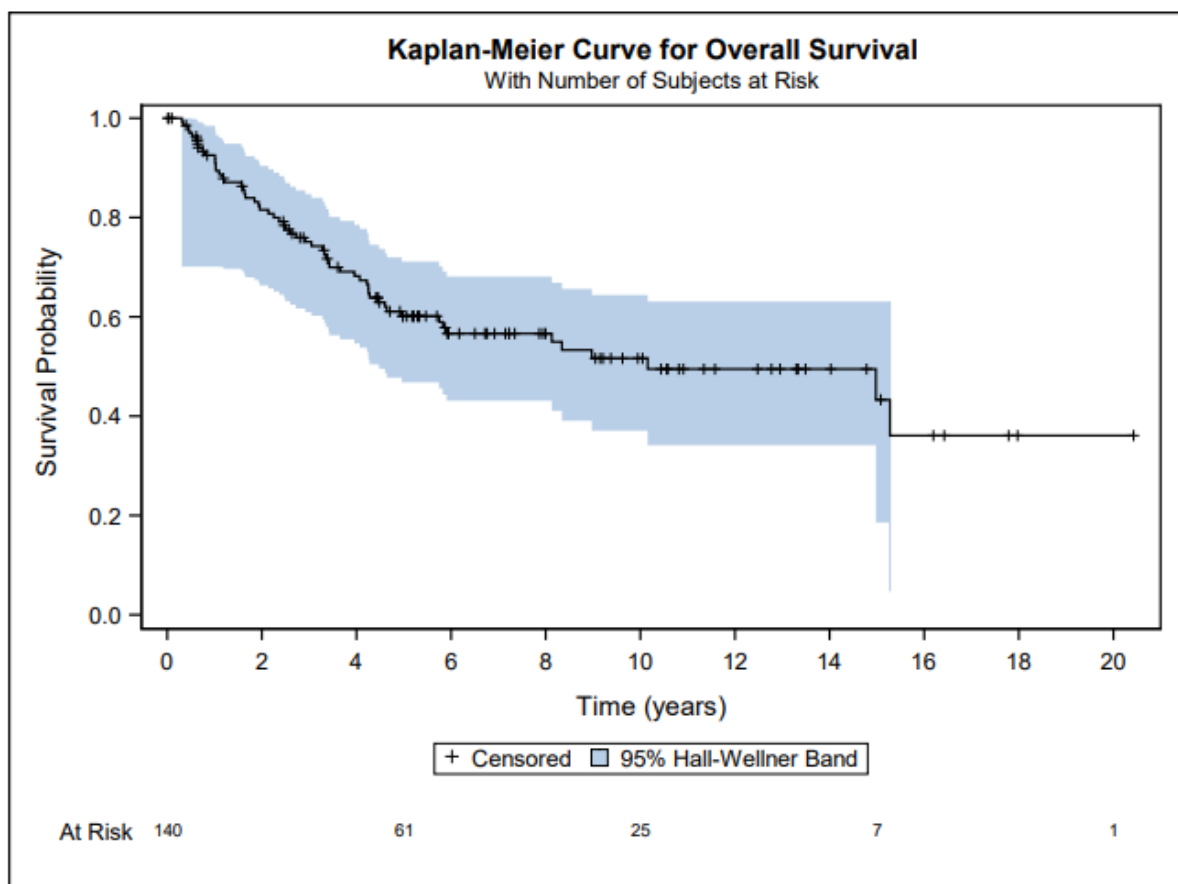


Figure 3-6 Kaplan-Meier Curve for OS

[illegible]

Years (%)	[19.2% - 53.3%]	[4.7% - 35.4%]								[1.1% - 32.0%]

Table 3-8 Summary of Overall Survival (OS) by Subtype

***At time of data review in August 2024.**

Parameter	Stage 1	Stage 2	Stage 3	Stage 4	Unknown Stage
Number of Patients Who Died	5	6	36	9	2
Number of Patients Last Known to be Alive	58	1	17	5	1
Median Time to Event (Years) [95% CI]	Inestimable	3.65 [0.3 – 5.9]	3.36 [2.6 – 4.6]	4.28 [1.0 – 9.0]	1.95 [0.8 – 2.0]
Overall Survival Rate at 2 Years (%)	94.9% [85.1% - 98.3%]	57.1% [17.2% - 83.7%]	75.7% [61.2% - 85.4%]	69.6% [37.8% - 87.4%]	0.0% [% - %]
Overall Survival Rate at 5 Years (%)	93.0% [82.3% - 97.3%]	28.6% [4.1% - 61.2%]	33.1% [19.9% - 46.9%]	34.8% [8.9% - 63.1%]	0.0% [% - %]
Overall Survival Rate at 8 Years (%)	93.0% [82.3% - 97.3%]	—	30.3% [17.5% - 44.2%]	23.2% [3.7% - 52.3%]	—
Overall Survival Rate at 10 Years (%)	—	—	26.0% [13.3% - 40.7%]	—	—
Overall Survival Rate at 15 Years (%)	—	—	10.4% [1.1% - 32.0%]	—	—

Table 3-9 Summary of OS by Stage

Parameter	Low Grade (G1&2)	High Grade (G3)	Unknown Grade
Number of Patients Who Died	7	50	1
Number of Patients Last Known to be Alive	35	32	15
Median Time to Event (Years) [95% CI]	Inestimable	4.48 [3.4 – 8.1%]	Inestimable
Overall Survival Rate at 2 Years (%)	87.5% [72.5% - 94.6%]	77.2% [66.3% - 85%]	90.9% [50.8% - 98.7%]
Overall Survival Rate at 5 Years (%)	84.5% [68.6% - 92.8%]	44.9% [33.4% - 55.7%]	—
Overall Survival Rate at 8 Years (%)	78.5% [57.6% - 89.9%]	39.4% [28.0% - 50.6%]	—
Overall Survival Rate at 10 Years (%)	—	34.5% [22.9% - 46.3%]	—
Overall Survival Rate at 15 Years (%)	—	23.9% [10.0% - 41.1%]	—

Table 3-10 Summary of OS by Grade

3.3.11 Genetic Testing Results

A total of 46 out of 140 patients (32.9%) underwent some form of genetic testing. Eight patients (5.7%) had no testing, another eight (5.7%) had an unknown testing status, two patients (1.4%) were awaiting testing, and 76 patients (54.3%) did not have data available or had blank records (**Table 3-11**). Among those tested, 14 patients (30.4%) had a specific CAV identified, while 32 (69.6%) did not. In the cohort of HGSOC patients, 50.8% (n=31) underwent genetic testing, with 11 (35.5%) showing a specific CAV. Among CAVs, 7 had *BRCA1* CAV (50.0%) and 4 had *BRCA2* CAV (28.6%), all within the HGSOC subgroup of patients. This represents a *BRCA1* prevalence of 15.2% and a *BRCA2* prevalence of 8.7% in this subgroup. Three out of ten patients with CCOC had a mismatch repair deficient (MMRd) cancer as determined by immunohistochemistry, all were confirmed to have Lynch syndrome (LS) (2 patients had *MSH6* and 1 had *MSH2*). Of note, the uptake of genetic testing increased over time, with the highest testing rate (44.4%) occurring between 2013 and 2015, and continued to remain high through 2020.

Characteristic	N (%)
Patients that underwent genetic testing	
Yes	46 (32.9%)
No	8 (5.7%)
Unknown Testing Result	8 (5.7%)
Awaiting Testing	2 (1.4%)
Data Not Available Blank	76 (54.3%)
Was a specific CAV identified?	
Yes	14 (30.4%)
No	31 (67.3%)
Unknown	1 (2.2%)
Genetic Testing in HGSOC Patients	
Yes	31 (50.8%)
No	3 (4.9%)
Unknown	5 (8.2%)
Awaiting Testing	1 (1.6%)
Data Not Available	21 (34.4%)
Was a specific CAV identified?	
Yes	11 (35.5%)
No	19 (61.3%)
Unknown	1 (3.2%)
Breakdown of Genetic Testing by Time Interval	
Before JUL 2005	8 (25.0%)
Between JUL 2005 - DEC 2007	16 (25.0%)
Between JAN 2008 - JUN 2010	15 (20.0%)
Between JUL 2010 - DEC 2012	24 (16.7%)
Between JAN 2013 - JUN 2015	27 (44.4%)
Between JUL 2015 - DEC 2017	27 (40.7%)
Between JAN 2018 - JUN 2020	23 (43.5%)

Table 3-11 YOC: Genetic Testing and Variant Detection

3.3.12 Analysis of Risk Factors

Correlation analysis was performed to assess associations between OC histologic subtype and selected clinical and lifestyle variables within the young-onset cohort (**Table 3-12**). Statistically significant positive correlations were observed between histologic subtype and parity ($r = 0.30$, $p = 0.001$), premenopausal status ($r = 0.28$, $p = 0.001$), history of miscarriage ($r = 0.22$, $p = 0.009$), and alcohol consumption ($r = 0.24$, $p = 0.004$). An inverse correlation was noted between subtype and height ($r = -0.34$, $p = 0.027$). These results suggest that variation in these factors may be associated with differences in tumour subtype across the cohort. However, the cross-sectional nature of the data limits any conclusions regarding causality, and these associations should be interpreted cautiously. Further multivariate or subtype-specific analyses would be needed to clarify the direction and clinical relevance of these relationships.

Variable	Correlation Coefficient*	P Value
Parity	0.3016	0.001
Premenopausal Status	0.2825	0.001
Height	-0.3409	0.027
Miscarriages	0.2162	0.009
Alcohol History	0.2397	0.004

Table 3-12 Correlation between OC subtype and variable

*Categorical variables use the Spearman correlation methods, continuous variables use Pearson correlation method.

3.4 Discussion

3.4.1 Prevalence of *BRCA1/2* PVs in a cohort of Irish OC patients

This is the largest study, to the best of the authors' knowledge, examining the nationwide prevalence of *BRCA* mutations in OC in the Republic of Ireland, reporting outcomes from parallel germline and tumour *BRCA* testing. Among patients who underwent *gBRCA* testing (455/535), 10.3% (47/455) were found to harbour a PV, 5.7% in *gBRCA1* and 4.6% in *gBRCA2* in our study. This rate aligns with a similar UK study involving 1,114 patients, which found *gBRCA1* at 3.6% and *gBRCA2* at 5.1%, resulting in a total *gBRCA* rate of 8.3% (301). A recent meta-analysis which included 55 studies in OC and over 48,000 patients, reports a higher prevalence rate of *gBRCA1/2* PVs in OC patients at 13.5% and 6.6% respectively, or a combined CAV rate of 21.1% (302). Comparatively, our findings suggest that, compared to wider international comparisons (288, 303-307), the observed rate of *gBRCA* CAV in our study is lower than anticipated at 10.3% (**Table 3-2**). Our *gBRCA1* rate (5.7%) in particular was lower than rates reported internationally (11.8% - 15.1%), and lower than the quoted average European rate of 15.1% (302). However, the alignment of these findings with a comparative real-world data from the UK (303), indicates that these variations may reflect broader trends rather than isolated anomalies. Supporting this, a US study reported the prevalence of *gBRCA* CAV in OC varied greatly between ancestry groups (288). They reported germline PVs in 25% of the whole cohort, however it was noted that this was skewed by high rates from those of Ashkenazi Jewish (39.9%) and Asian heritage (26.5%). They reported a European rate of 10%, which aligns with our findings (288). Similarly, the higher rate of *sBRCA* PVs observed in this study (9.5%) and the UK study (10.4%)(301), compared to the meta-analysis data (7.6%)(302), suggests that the specific populations under investigation may possess unique genetic characteristics or environmental factors that impact the prevalence of somatic mutations. The combined germline and somatic *BRCA* rate reported in HGSOc subgroup in this study (n=462, **Table 3-3**) is 21.8%, which is similar to a previous study in an Irish cohort of HGSOc patients (n=70), which found an overall *BRCA1/2* mutation rate of 25.7% (295).

There were 47 unique *gBRCA* CAV detected in the *BRCA* genes, 26 unique *BRCA1* CAV and 21 unique *BRCA2* PV. Among *BRCA1* carrier patients in this cohort, the most common mutation identified was c.5266dupC, a frameshift mutation, present in 1.25% of the patients. This particular *BRCA1* PV, c.5266dupC, is one of the most frequently observed mutations across Europe (308) and is notably prevalent in the Ashkenazi Jewish population (309). Its prevalence in this study aligns with broader European data, reflecting its widespread distribution. In Ireland, this mutation has been documented across various regions, including the eastern, midland, and southern parts of the country. Additionally, the deletion, c.1175_1214del, was identified in four patients, all originating from referral facilities in the south of Ireland. This mutation creates a premature translational stop signal in the *BRCA1* gene, leading to an absent or disrupted protein product, thereby causing a loss-of-function variant in *BRCA1* (310). This variant is frequently reported in individuals of British and Irish

ancestry (311, 312). Among *BRCA2* PVs, the most prevalent mutation identified in our study was *c.4398_4402del*, found in four patients (0.83% of the cohort), all originating from referral facilities in the south of Ireland. This mutation is located in coding exon 10 of the *BRCA2* gene and results in a frameshift, leading to a truncated, non-functional protein (313). This sequence change creates a premature translational stop signal (*p.Leu1466Phefs*2*) in the *BRCA2* gene and is expected to result in an absent or disrupted protein product (310).

This study has several notable limitations. Firstly, it did not encompass comprehensive clinical data on elements such as testing uptake, tumour characteristics, family history, ethnicity, previous treatment histories, treatment responses, and long-term follow-up outcomes. Such data was beyond the scope of this national retrospective study, thereby limiting a thorough understanding of the broader contextual factors influencing the study findings. The challenges in accessing this data underscore issues related to underdeveloped electronic patient records, fragmented health information technology systems across the country, and the absence of dedicated registries for patients with cancer predisposing syndromes that could systematically document clinical outcomes (314). These constraints pose a significant barrier to compiling comprehensive epidemiological data within specific disease cohorts within Ireland, highlighting areas for improvement in healthcare data infrastructure and research methodologies.

A second limitation, as mentioned earlier, is that this study did not include individuals who had previously undergone *BRCA* testing due to familial screening or prior malignancies such as breast cancer. This exclusion could particularly impact the identification of *gBRCA* results, which are often detected through screening programs for inherited risks. Moreover, Ireland's dual public-private healthcare system meant that patients who opted for private testing were not included in this analysis. Lastly, patients who were not referred for testing by their clinicians were also not captured in this study.

A third limitation of this study is its exclusive focus on *BRCA1/2* PVs in OC, despite the broader relevance of Homologous Recombination Deficiency (HRD). It is recognized that while approximately 50% of HGSOC cases exhibit HRD dysfunction, only 30% of these cases are linked to *BRCA1* or *BRCA2* mutations stemming from germline, somatic, or epigenetic alterations (3). The emerging recognition of HRD as a significant predictive biomarker for both HGEOC and HGSOC suggests a wider application for genetic testing strategies (315). The pivotal role of the HRR pathway in DNA repair and the sensitivity of HRD-positive tumours to PARP inhibitors, further highlights the importance of integrating HRD testing into clinical practice for advanced OC patients, reflecting the evolving landscape of precision medicine (75).

Future work in this area is essential to further elucidate the prevalence and implications of *BRCA* PVs in OC. Our group has applied for ethics approval and secured industry-sponsored funding to establish

a pilot study focused on creating a cancer predisposing genetic alteration registry 'CAmPaiGn Study', (Project ID 3824). This initiative aims to systematically collect and analyse demographic and genetic data, providing a comprehensive understanding of the genetic landscape within our population. To enhance the study's relevance and effectiveness, patient and public involvement (PPI) are being engaged with to gain insights into the demographics and needs of the patient community. This collaboration will help address critical questions regarding the characteristics of individuals with *BRCA* pathogenic variants, ultimately guiding future screening, prevention, and treatment strategies in OC. Through these efforts, the study aims to contribute valuable data that can inform clinical practice and improve patient outcomes in Ireland.

From this study, cost-effectiveness and turnaround time (TAT) of integrated testing vs. reflex testing strategies for *BRCA1/2* mutations in OC patients was modelled. The retrospective analysis of 292 patients demonstrated that paired testing identified 30 germline *BRCA* PVs, all of which were concordant with tumour *BRCA* PVs. In contrast, of the 262 germline-negative patients, 9.5% were found to have somatic *BRCA* PVs. Integrated testing, which was the most comprehensive, detected all *BRCA* mutations but at a cost of €408,800, compared to reflex testing models—tumour-first and germline-first—which were more cost-effective, with prices of €219,200 and €251,600, respectively. Reflex testing models offered significant cost reductions of 46.4% and 38%, respectively, while also yielding shorter turnaround times (26 days for reflex testing vs. 35 days for integrated testing). However, it is important to note that reflex approaches were associated with the risk of missing two large genomic rearrangements (LGRs) and 9.5% of somatic cases. These findings suggest that while reflex testing strategies are more cost-effective and quicker, integrated testing remains critical for ensuring complete detection of both germline and somatic mutations, which is vital for optimal patient management and treatment planning. The choice between integrated and reflex testing strategies may depend on the healthcare context, where balancing cost-effectiveness, diagnostic accuracy, and timely decision-making is essential for improving patient outcomes.

3.4.2 Survival Trends and Pathologic Features of OC in Young Women

This study presents a comprehensive single-institution assessment of YOC, offering a detailed analysis of clinicopathological features, survival outcomes, and genetic profiles in patients under 50 years. As anticipated, survival outcomes were significantly influenced by both stage and histological subtype. A key finding is the high proportion of early-stage diagnoses in this cohort (45% at stage I), in contrast to the typically late-stage presentation seen in older populations. This may reflect underlying differences in tumour biology, as younger patients are more often affected by 'Type I' OC, which tend to grow larger and present with symptoms earlier (39). Despite this, HGSOC, a 'Type II' OC, remained the most common subtype and was associated with the poorest survival, aligning with previous data that highlight its aggressive nature and late presentation even in younger women (50, 292). Our study also revealed demographic differences compared to population-based registries such as the SEER database, which reports that approximately 30% of OC cases occur in patients aged 54 or younger (284). In contrast, our study found this figure to be 15%. This discrepancy may be attributed to the absence of paediatric patients in our study, as well as geographical, ethnic, and genetic differences. The correlation of certain lifestyle and reproductive factors with histologic subtypes underscores the importance of individualised risk profiling in young women presenting with OC.

In this study, genetic testing revealed a significant prevalence of PVs, particularly *BRCA1* and *BRCA2* mutations in HGSOC, aligning with existing literature (316). *BRCA1* and *BRCA2* CAV carriers have an estimated 40–50% and 10–20% risk of developing EOC by age 70, respectively (28), with *BRCA1*-associated EOC occurring at an earlier age compared to sporadic cancers, typically in the mid-40s (19). Notably, 35.2% of those tested in the HGSOC subgroup harboured a PV. This highlights the importance of consistent genetic counselling in OC care, especially given the proven efficacy of PARPi in treating *BRCA*-driven OC (8-12). None of the patients in this cohort underwent HRD testing, likely due to its limited availability at the time of diagnosis and evolving clinical guidelines. During the study period, routine HRD testing was not yet integrated into national practice. However, more recent guidelines from the European Society for Medical Oncology (ESMO) and the American Society of Clinical Oncology (ASCO) now recommend HRD testing to guide treatment decisions in patients with high-grade serous ovarian cancer, particularly in the context of PARP inhibitor therapy (52, 286).

Similar to patients with a *gBRCA* PV, the average age of diagnosis of OC is typically younger in women with LS with 25%–30% of the women diagnosed before the age of 40 (317); these individuals also carry a lifetime OC risk of 6–8%, compared to 1.4% in the general population (19). Histologically, LS-associated OCs are primarily ENOC (40%), HGSOC (25%), and LGSOC (11%) or CCOC (6%) (317). Of note, three of ten (30%) patients with CCOC in our study had MMRd tumours. Despite immunotherapy demonstrating remarkable and durable responses in patients with MMRd tumours across multiple cancer types, supporting its role as a site-agnostic therapeutic strategy (318-320), clinical data remain limited on its use in this cohort due to the rarity of MMRd in CCOC. Even so,

recent emerging evidence appears to support the role for immunotherapy specifically for this group. In the phase II PEACOC trial, pembrolizumab demonstrated clinical activity in patients with previously treated MMRd clear cell gynaecological cancers, 85% of which were CCOC, with a disease control rate of 42% and an ORR of 25% (321). Similarly, a phase II study of nivolumab in MMRd or hypermutated gynecologic cancers included two CCOC patients—both of whom achieved durable responses (322). Ongoing trials continue to evaluate checkpoint inhibitors in this setting (323). These findings suggest that while limited, the current data indicate meaningful responses to immunotherapy in MMRd CCOC, warranting broader germline and somatic testing in non-serous subtypes. These findings support the need for histology-driven testing strategies that extend beyond *BRCA*, which perhaps need to be tailored to the unique characteristics of the YOC population.

Beyond genetic predisposition, treatment considerations for YOC are uniquely complex. Even when a CAV is identified and a targeted therapy option is available, long-term toxicity, as well as potential impacts on hormonal health and psychosocial well-being, are key considerations for this population (50, 324). Younger patients may face prolonged or even lifelong exposure to anti-cancer treatments, raising concerns that cumulative toxicity and adverse effects could outweigh the benefits (299). For instance, maintenance treatment with PARPi remains the standard of care for platinum-sensitive HGSOC. However, ongoing studies are examining the optimal treatment durations and whether cut-off points should be defined to balance efficacy with long-term safety (325). Similarly, the optimal duration of immune checkpoint blockade is under investigation, with research focused on determining the appropriate length of treatment to achieve an optimal balance between safety and efficacy (326). Defining evidence-based cut-off points will be particularly relevant for younger patients, given the longer duration of potential exposure to these drugs.

The balance between oncologic control and fertility preservation is also key concern, especially in early-stage or low-grade diseases (299). Fertility-sparing surgery may be appropriate in select cases, but this must be weighed against the risk of recurrence and the biological behaviour of the tumour. The recent ASCO guideline update (2024) highlights the importance of early fertility preservation counselling as part of routine care (327).

This retrospective study has several limitations. Genetic testing was not uniformly applied across the cohort, which could introduce selection bias, as patients with stronger clinical or familial risk factors may have been more likely to undergo testing. As a result, the prevalence of PVs observed may not fully represent the broader Irish YOC population. The genetic analysis was also limited to known PV, rather than comprehensive genomic profiling or panel testing (i.e., HRD), which restricts the depth of the findings. Additionally, the study was conducted at a single national referral centre, which may limit its generalizability, especially in healthcare systems with different demographic or genetic backgrounds. While important aspects such as psychosocial outcomes, fertility preservation

strategies, and quality-of-life measures were not assessed in this study, these factors remain highly relevant in YOC research. The absence of paediatric patients (defined as <16 years) in the cohort may introduce a degree of selection bias; although rare, this age group constitutes a small but distinct subset of the young-onset ovarian cancer population.

Despite these limitations, the study's strengths lie in its comprehensive evaluation of YOC at a high-volume tertiary care centre over an 18-year period, incorporating clinical data, histopathology, genetic testing, and survival outcomes. The cohort's diversity in histologic subtypes, ranging from high-grade serous to rare germ cell and stromal tumours, provides a broader and more nuanced perspective compared to studies focusing exclusively on EOC. Survival analyses stratified by stage and grade add clinical relevance, and the increasing trend of genetic testing uptake underscores the growing shift toward personalized medicine (**Table 3-11**). These strengths contribute valuable insights that can inform targeted interventions and early detection strategies for young women with OC.

Designing and Launching a Pilot Registry for Cancer- Predisposing Genetic Mutations

Chapter Four

4 Designing and Launching a Pilot Registry for Cancer-Predisposing Genetic Mutations

4.1 Introduction

As outlined in the previous chapter, there is a lack of published data on cancer-predisposing genetic alterations among patients treated in Ireland. Despite Ireland's strong economic standing, its eHealth infrastructure remains significantly underdeveloped (328). In 2023, Ireland ranked lowest in the European Union for digital health integration, with only 11% implementation, highlighting its position as a clear outlier in healthcare digitisation (**Figure 4-1**) (329). This limited digital infrastructure constrains the integration of genomic data into clinical care, despite the transformative potential of germline genetic testing to identify individuals at elevated cancer risk and to inform targeted prevention strategies (94, 95).

Health scores in the EU: access to eHealth records

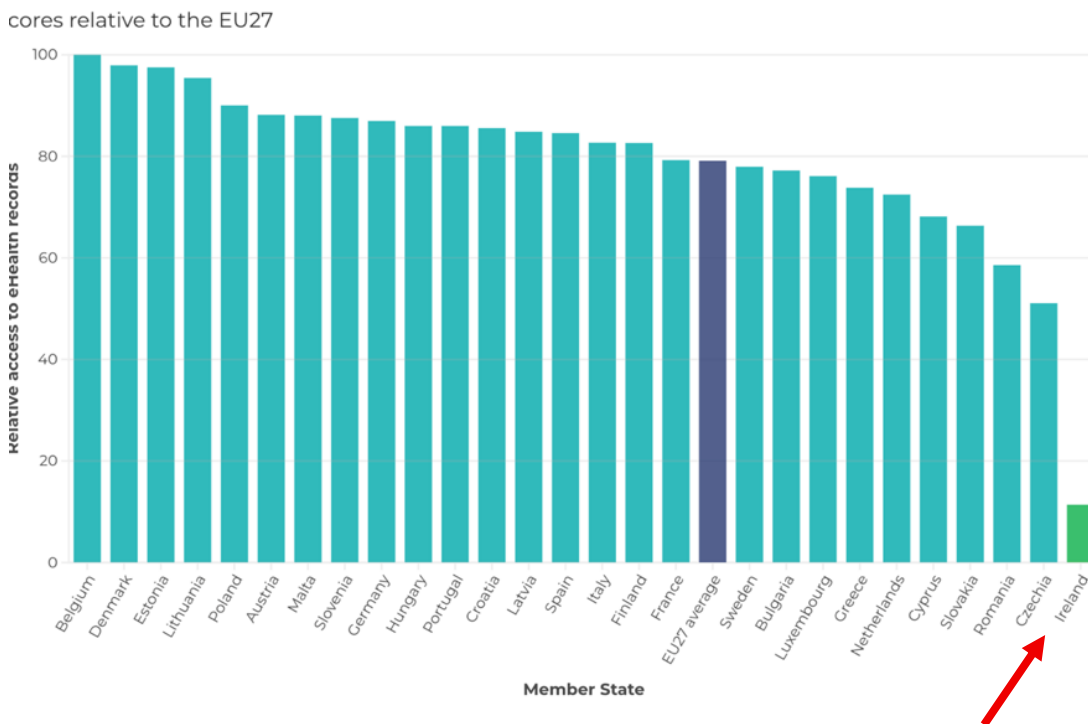


Figure 4-1 eHealth Scores in the EU : access to eHealth records

The absence of a national registry for genetic cancer data poses significant challenges for health forecasting and clinical decision-making. For example, patients with genetic PV, such as *BRCA1/2*, may benefit from targeted therapies (e.g., PARP inhibitors), yet the prevalence of such mutations across various cancer types (e.g., pancreatic, prostate, breast, ovarian) in Ireland remains poorly reported. As a result, decision-making around treatment access, budgeting, and resource allocation becomes difficult. Moreover, there is a notable lack of data on individuals who carry cancer-predisposing genetic alterations but have not yet developed cancer. Understanding the timeline between the diagnosis of a cancer predisposing CAV and the development of cancer, as well as

patient engagement in screening and prophylactic interventions, is crucial for refining preventive strategies (330). Yet, much of the available data on these aspects is fragmented and stored in isolated centres, further complicating efforts to create national or international comparisons. The dual healthcare system in Ireland, comprising both public and private sectors, further exacerbates this issue. Without a national registry, it is challenging to capture data on all patients, particularly those who seek genetic testing through private channels, such as privately paid for Foundation One testing, thus limiting the comprehensiveness of data reporting. This lack of centralized genetic information hampers the ability to create a unified national forecast or strategy for cancer prevention, treatment, and budgeting.

The National Strategy for Accelerating Genetic and Genomic Medicine in Ireland, published in 2022, recognizes the need for developing national registries for patients with genetic alterations that predispose them to cancer (94). This strategy highlights the importance of integrating genomic data into clinical practice, especially given the increasing role of genomics in personalized medicine. Beyond infrastructure, training and workforce development within the HSE also play crucial roles in meeting future goals for genetic services in Ireland. A 2022 survey highlighted gaps in postgraduate training in cancer genetics across multiple specialties (95), indicating a need for more comprehensive education and training programs. The HSE's 2019 review of the Clinical Genetics Medical Workforce emphasized the shortage of trained geneticists and the need for strategic workforce planning (96). The strategy further emphasises the necessity of building registries that are both comprehensive and inclusive, a goal that can only be achieved through meaningful collaboration with patients and the public. The inclusion of Public and Patient Involvement (PPI) has been emphasized to ensure that these registries reflect diverse patient experiences and needs while facilitating comprehensive data collection and management (95).

These challenges highlight the pressing need for the establishment of a national registry to collect and manage genetic data related to cancer predisposition. While the creation of such a registry is essential, it also presents significant logistical and technical hurdles, many of which are like the challenges that currently hinder the integration of data across hospitals and healthcare providers in Ireland. The introduction of stricter data protection regulations by the EU, such as the General Data Protection Regulation (GDPR), presents additional complexities concerning data ownership, data controllers, and patient privacy.

Against this backdrop, this chapter explores the feasibility and early outcomes of establishing a pilot registry for patients with cancer-predisposing genetic alterations in Ireland. It considers the infrastructural, clinical, and PPI-related components required for national-scale implementation and highlights early insights that may guide future registry development.

4.2 Aims and Objectives

4.2.1 General Aims

The aim of this chapter is to report on the development and launch of the CAmPaiGn Study pilot registry at St. James's Hospital, focusing on collecting and analysing genetic and demographic data for patients with any cancer predisposing genetic alteration.

4.2.2 Hypothesis

It was hypothesised that establishing a pilot registry for cancer-predisposing genetic alterations at St. James's Hospital is both feasible and informative, enabling systematic data collection and patient engagement that will support future genomic research and service development. Furthermore it was hypothesised that structured involvement of PPI representatives would contribute valuable perspectives to guide the design and development of the registry.

4.2.3 Objectives

The objectives of this chapter are as follows:

- To establish a pilot observational database for patients harbouring any cancer-predisposing genetic alterations at St. James's Hospital (SJH).
- To assess the feasibility and potential operational impact of initiating a cancer predisposition genetic observational database in the Irish population.
- To evaluate clinicopathological characteristics and therapeutic patterns of those enrolled in the study

4.3 Results

4.3.1 Conceptualising of the CAMpaiGn Study Registry

The conceptualisation of the CAMpaiGn registry arose from the recognition that, unlike many international counterparts, Ireland lacks a centralised system to capture and analyse germline genetic data, including *BRCA* status. This deficiency is exacerbated by fragmented and often incompatible electronic health record (EHR) systems across public and private institutions, and the absence of a national registry for hereditary cancer-related mutations. Consequently, there remains a reliance on international datasets, despite known variation in the prevalence and spectrum of cancer-predisposing genetic alterations by ancestry and geography (288). Collectively, these factors (**Figure 4-2**) contribute to a fragmented and underdeveloped landscape for hereditary cancer genomics in Ireland, which ultimately impacts patient care and underscores the need for coordinated national solutions.

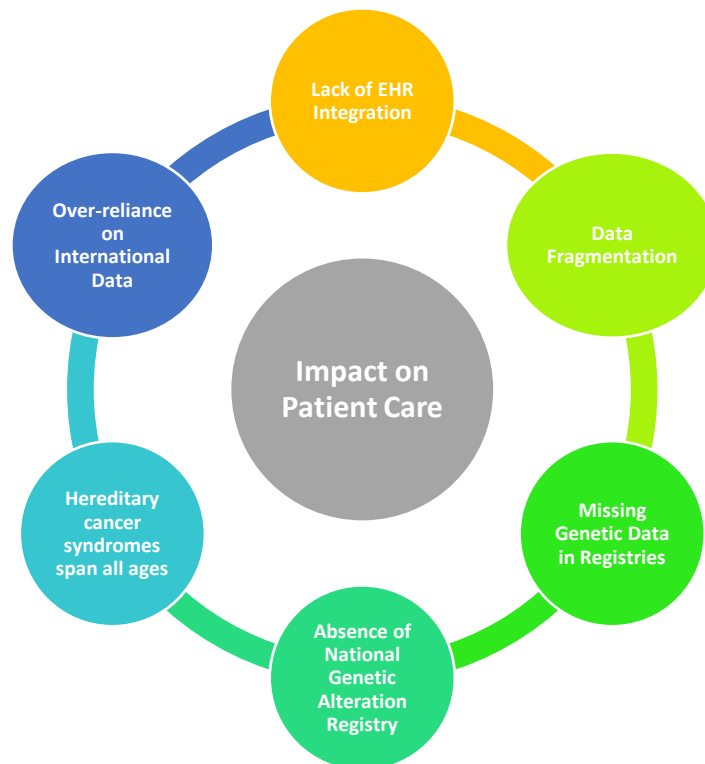


Figure 4-2 Barriers to Genetic Data Integration in Ireland

To address the critical gap in Ireland's infrastructure for collecting and managing cancer-related genetic data, a pilot registry for cancer-predisposing genetic alterations was conceptualised and established at St. James's Hospital. The CAMpaiGn Study was designed to systematically collect and analyse data from patients harbouring any cancer-predisposing genetic alteration. Supported by industry-sponsored funding and full ethical approval (Project ID 3824), the project aims to provide a comprehensive understanding of the genetic landscape among Irish patients with hereditary cancer syndromes.

4.3.2 Information Gathering – Insights from Conferences and Networking

As part of the information-gathering process for the CAmPaiGn Study registry, attendance at relevant conferences and networking events was crucial. One significant event was *The Future of Patient Registries in Ireland*, hosted by the Cystic Fibrosis Registry of Ireland in association with Health Research Charities Ireland (HRCI). Held on February 2nd, 2023, at the Ashling Hotel in Dublin (331). This in-person event brought together key stakeholders involved in patient and disease registries across Ireland. The event provided valuable insights into the challenges and opportunities associated with operating registries in the Irish context, particularly focusing on the sustainability of such initiatives. Discussions at the conference helped refine the approach for the CAmPaiGn Study registry, offering practical knowledge on registry management, data integration, and long-term viability in the Irish healthcare system. Furthermore, the Irish Health Research Forum, titled "*Is it Time to Get Serious About Patient Registries?*", hosted by Health Research Charities Ireland on May 15th, 2025(332), further built upon these discussions, continuing to explore the evolving role of patient registries in Ireland's health research landscape. The structured discussion groups addressed four core themes: integration and interoperability, highlighting the need for harmonisation with national systems and alignment with the European Health Data Space; coordination and investment, emphasising the importance of strategic governance structures and sustainable funding; co-design and participation, which reinforced the value of involving patients and service users in shaping registry purpose and data use; and impact and innovation, which explored how registries can evolve to generate real-world evidence and harness emerging technologies, such as artificial intelligence, while maintaining public trust. These discussions directly informed the strategic planning and design of the CAmPaiGn Registry by identifying priority areas for development, stakeholder collaboration, and long-term sustainability.

4.3.3 Identifying Gaps in Current Genetic and Cancer Registry Data

A key challenge in Ireland is the limited use of EHR across hospitals, hindering comprehensive data collection. Initially, the concept of developing a national registry was inspired by the model of the Cystic Fibrosis Registry (333), with an initial focus on creating a *BRCA*-specific registry. However, following discussions with key stakeholders (outlined below) and from reviewing the literature (90, 95), it became evident that such a registry would exclude other important groups affected by cancer-predisposing genetic alterations. Moreover, while the initial focus was on the adult population, these consultations highlighted the need to include the Adolescent and Young Adult (AYA) group as a crucial demographic.

4.3.4 Research Existing Registries

There are other specialised registries in Ireland, such as the Cystic Fibrosis Registry (333), Renal Transplant Registry (91) and the emerging National Chronic Disease Registry (92) which represent important steps toward the systematic collection of healthcare data. While Ireland has a well-

established National Cancer Registry (NCRI) (83), it is worth noting that the NCRI does not currently collect genetic data, highlighting a significant national gap in understanding the genetic factors underlying cancer predisposition (83, 94). Additionally, certain medical specialties have created local disease-specific registries, which are often developed through collaborative efforts within their respective networks such as the Rare Kidney Disease & Vasculities Registry (334). Ireland’s involvement in European Reference Networks further enhances its ability to contribute to international rare disease registries (93), fostering greater collaboration across borders and improving the understanding of rare conditions, including genetic disorders that predispose individuals to cancer. In parallel with a review of the literature on registries in Ireland, informal emails and phone calls with the CEO of the Cystic Fibrosis Registry provided invaluable insights and greatly facilitated information gathering. These discussions offered practical guidance on navigating compliance and ethics processes, approaches to secure data collection and storage, strategies for achieving patient buy-in, and advice on the day-to-day management and operational sustainability of a disease-specific registry.

4.3.5 Engagement with Stakeholders

Engagement with established stakeholders was crucial in garnering existing expertise and insights specific to the Irish healthcare and research setting, ensuring the development of the CAmPaiGn Study Registry was informed by practical, field-relevant knowledge. **(Figure 4-3).**



Figure 4-3 Stakeholder Engagement in Registry Development

(i) Clinical Care Pathways

Clinical leads in paediatric, adolescent, and adult genetics and oncology were engaged through

informal interviews and information-gathering meetings conducted in person or via Zoom. These included Prof Owen Smith (Paediatric Haematology), Prof Andrew Green (Clinical Genetics), Dr Scheryll Akler (Adolescent and Young Adult Oncology), Prof Karen Cadoo (Medical Oncology and Cancer Genetics), Prof David Gallagher (Medical Oncology, National Lead for Cancer Genetics), and Assistant Professor Rosie O'Shea (Principal Genetic Counsellor). Their input provided detailed knowledge of patient care pathways and advocated for a registry design that would accommodate both adult and paediatric populations, thereby supporting inclusive and comprehensive data capture. Clinical specialists from both adult and paediatric genomic services contributed further insights into current testing practices, reporting structures, and the integration of genomic data into clinical workflows. These contributions were instrumental in shaping a registry framework that aligns with real-world clinical practice and supports long-term utility in both oncology and genetics settings.

(ii) Supportive Care Services

A clinical psychologist with expertise in psycho-oncology, Dr Sonya Collier (BA), was identified and introduced by the clinical team as an invaluable contributor to the registry development process, given her dual role in psycho-oncology at St. James's Hospital (SJH) and Children's Health Ireland (CHI). Dr Collier, who sees both adult and paediatric patients, participated in informal discussions which were conducted in person and via Zoom. She provided valuable insights into addressing psychosocial distress in individuals with hereditary cancer syndromes, drawing on recent Irish research that highlights the emotional burden experienced following the identification of a cancer-predisposing pathogenic variant (335, 336). Her input ensured that the registry design extended beyond clinical and genomic data to incorporate the importance of access to psychological support services, thereby promoting a more holistic and patient-centred approach to care.(335, 336)

(iii) Governing Bodies and Compliance

The National Cancer Control Programme (NCCP) was engaged as a key stakeholder to ensure alignment between the CAMPaIGn Registry and national cancer control strategies, particularly in the areas of genetic risk assessment, prevention, and service planning. Engagement included participation in an invite-only national virtual forum hosted by the NCCP, during which Dr. Ciara McNevin delivered a presentation titled "*Building a Registry for Patients with Cancer-Predisposing Genetic Alterations: Our Journey and Insights*" on 11th April 2024. This presentation formed part of a broader NCCP educational session and facilitated structured discussion and feedback from national stakeholders, including representatives from the NCCP, Clinical Genetics, Oncology, Public Health, and the Health Service Executive (HSE) policy and data governance teams.

(iv) Ethical, Legal, and Data Management Considerations

The development of a national registry for cancer-predisposing genetic mutations involves complex ethical and legal considerations, particularly when operating across multiple healthcare sites. One of the key challenges was determining the appropriate data controller and ensuring full compliance

with data protection legislation. Early and ongoing engagement with the Data Protection Officer (DPO) at St. James's Hospital (SJH) was critical in navigating these complexities. The DPO provided essential guidance on data governance, privacy, and security, and played a central role in shaping the registry's approach to consent procedures, secure data storage, and cross-institutional data sharing.

This support was instrumental in launching the local pilot registry in compliance with institutional policies and national data protection standards, including the General Data Protection Regulation (GDPR)(337), the Health Research Regulations (HRRs) 2018 (as amended) (338), and the St. James's Hospital Data Protection and Information Governance Framework (339). The management of personal information within the registry is governed by the Data Protection Acts 1988 to 2018 and the European General Data Protection Regulation 2016 (GDPR), ensuring that all data are handled lawfully, fairly, and transparently.

Ethically, the registry prioritises patient autonomy, informed consent, and responsible data use. Engagement with Dr. Sadhbh O'Neill, Research Ethics and Clinical Trials Manager at SJH, was key in navigating these concerns. Dr. O'Neill emphasised the importance of clear communication with participants regarding how their data would be used, the associated risks and benefits, and their right to withdraw consent at any time. This ethical framework fosters participant trust and ensures that the registry operates in a manner that respects patient dignity and autonomy.

In parallel, consultation with hospital ethics committees ensured that all aspects of the registry's informed consent process were robust, transparent, and ethically sound. These interactions ensured that patient data would be used responsibly, ethically, and in accordance with both legal requirements and best practice guidelines in health research.

(v) Advocacy Groups and Non-Profit Organizations

Patient advocacy groups, including BRCA Ireland and Lynch Syndrome Ireland, highlighted the importance of addressing the specific needs of high-risk populations, ensuring the registry reflects the requirements for screening, surveillance, and treatment of BRCA mutations and Lynch Syndrome. Additionally, collaborations with the Irish Cancer Society and the Marie Keating Foundation supported public engagement and funding strategies, further strengthening the registry's foundation.

(vi) PPI

A critical component of the CAmPaiGn Study is the active involvement of Patient and Public Involvement (PPI), ensuring that the registry is shaped by those directly affected by hereditary cancer syndromes. By engaging patients and the wider public, the registry is being developed with direct input from individuals affected by cancer-predisposing genetic mutations, enhancing its relevance, accessibility, and clinical utility. PPI input was gathered through several practical mechanisms:

- **Informal conversations with individuals with lived experience**, including patients and family

members, which provided valuable insight into the emotional, clinical, and logistical challenges they face;

- **Email correspondence with advocacy groups** such as BRCA Ireland and Lynch Syndrome Ireland, which helped identify key priorities for inclusion in the registry, including preferences for communication, consent, and support;
- **Networking at events**, including the Marie Keating Foundation's BRCA-focused seminar, which facilitated broader engagement with patient advocates and healthcare professionals working in hereditary cancer care.

These interactions helped ensure the registry reflects the lived experiences and priorities of the patient community. Through this approach, the study aims to establish a foundation for improved genetic risk stratification, screening, risk reduction, and treatment strategies—particularly in ovarian cancer—and to generate population-specific data on the implications of *BRCA* CAVs in Ireland.

4.3.6 Finalising the Registry Framework and Rationale

Through engagement with key stakeholders, several core structural elements emerged as critical for the design of the CAmPaiGn Study Registry. It was evident that a comprehensive registry for all cancer-predisposing genetic alterations was necessary, encompassing individuals both *with and without* a current or prior cancer diagnosis. Additionally, the registry needed to span both adolescent and young adult (AYA) populations, as well as the adult population, ensuring inclusivity. Patient and public involvement (PPI) was also highlighted as essential in shaping the registry to meet the needs of all stakeholders. However, the complexity of establishing such a registry encompassing everything outlined above on a national scale, was beyond the resources, time, and scope of this PhD project. Consequently, it was decided to initiate a pilot study focused on a localized registry for all cancer-predisposing genetic alterations at SJH. To guide data collection, a Case Report Form (CRF) was developed (**Appendix 2**), outlining pertinent data points to be collected while adhering to the principle of data minimization to ensure only relevant and necessary information is gathered.

4.3.7 IT / Data Considerations

Establishing the legal ownership of the data was a critical first step in the development of the registry, ensuring clarity regarding responsibility for its protection and management. A dedicated data manager was employed to oversee data collection processes, ensuring consistency, accuracy, and adherence to ethical and regulatory standards. Data were collected using standardised case report forms and securely transferred to the registry database, with appropriate validation and quality control procedures implemented.

To safeguard patient privacy, all data were stored on secure servers with robust encryption protocols to prevent unauthorised access. The registry also adhered to the principle of data minimisation, ensuring that only essential data required for the study's objectives were collected, stored, and

processed. These measures aligned with ethical standards and data protection legislation governing the handling and retention of sensitive health information.

These considerations were comprehensively addressed in the Data Protection Impact Assessment (DPIA), which was reviewed by the Data Protection Officer (DPO) and submitted to the ethics committee as part of the governance process. This rigorous approach to IT infrastructure, data governance, and collection procedures was integral to maintaining participant confidentiality and preserving the integrity of the registry.

4.3.8 Consolidating the Conceptual Framework

This collaborative approach leveraged the experience of professionals across various disciplines, enabling the identification and resolution of challenges related to registry development, data integration, and participant engagement. Finalizing the conceptual framework involved synthesizing input from key stakeholders and aligning it with the study's objectives. This process ensured that the registry's design would effectively capture relevant data while addressing the specific needs of cancer-predisposing genetic alterations. The framework provided a structured approach to guide data collection, management, and analysis, ensuring consistency and clarity in how the study would be conducted. By integrating insights from clinical experts, genetic counsellors, patient advocacy groups, and ethical guidelines, the framework laid a solid foundation for the development of a robust and comprehensive registry.

4.4 Development of the Pilot Registry

4.4.1 Study Design

4.4.1.1 Sample Size

The clinical genetics service currently reviews approximately 1,200 patients annually, with projected increases to 1,500 patients in the upcoming year and a sustained annual growth rate of 10–20% thereafter. Given these projections, it is estimated that between 4,000 and 4,500 patients will be seen across the three-year study period (2025–2028).

A target accrual of 1,000 patients over three years—representing approximately 22–25% of the total eligible population—is considered appropriate for the exploratory nature of this pilot study.

This sample size is not intended for hypothesis testing but is sufficient to:

- Assess the feasibility of registry processes (e.g. consent, data capture, follow-up),
- Identify the range and frequency of cancer-predisposing genetic alterations encountered in the Irish setting,
- Evaluate the representativeness and completeness of registry data, and
- Inform power calculations and methodological refinement for future national-scale implementation.

Given that this is a pragmatic, feasibility-focused pilot, formal power calculations are not strictly required. However, the inclusion of 1,000 patients allows for meaningful subgroup exploration (e.g. *BRCA*, Lynch Syndrome cases etc) and provides an adequately sized dataset to test data workflows, governance procedures, and reporting outputs before scaling to a national registry.

4.4.1.2 Inclusion Criteria

In line with the registry's aim to capture real-world data on participants with a cancer predisposing genetic alteration, the following inclusion and exclusion criteria were applied to guide participant eligibility:

- Inclusion criteria
 - Persons who are attending the clinical genetics department in St James's Hospital
 - Patients able to give signed and written informed consent
 - Patients over 18 years of age
- Exclusion criteria
 - Patients who do not give consent

4.4.1.3 Outline how participants will be identified

- **Educational Seminar:** Genetic counsellors involved in the study will first attend a

comprehensive educational seminar. This seminar will cover all aspects of the study, including eligibility criteria, the process of obtaining informed consent, and the ethical considerations involved.

- **Identification of Eligible Participants:** Following the seminar, genetic counsellors will identify eligible patients during their routine consultations in the genetic clinics. These patients will be selected based on the predefined inclusion criteria outlined in the study protocol.
- **Providing Participant Information:** Once an eligible patient is identified, the genetic counsellor will provide them with a Participant Information Leaflet (PIL) (**Appendix 3**). This document explained the study's purpose, procedures, and any potential risks or benefits, ensuring that patients have all the necessary information to make an informed decision.
- **Obtaining Consent:** After giving the patient sufficient time to consider their participation, the genetic counsellor will obtain informed consent from those who agree to participate. This process will be conducted in accordance with ethical guidelines to ensure that consent is voluntary and well-informed.
- **Data Management:** Once consent is obtained, the genetic counsellor will forward the participant's information to the data manager. The data manager will then complete a CRF for each participant, documenting all relevant data for the study.

This structured approach ensures that participants are identified, informed, and enrolled in the study in a manner that respects their autonomy and adheres to ethical standards.

4.4.1.4 Outline how participants will be recruited

Participants were recruited through a structured, multi-step process led by trained genetic counsellors within routine clinical genetics consultations. Eligible patients were introduced to the study during their clinic visit and provided with a PIL outlining the study's purpose and procedures. Patients were then given adequate time to consider participation, followed by a follow-up discussion—either in person or via phone—to answer any questions and ensure full understanding. If the patient agreed to participate, informed consent was obtained in line with ethical guidelines. Upon consent, enrolment details were forwarded to the data manager, who completed the Case Report Form (CRF), formally registering the participant.

Consent was obtained either in person or remotely, depending on patient preference. In-person consent involved direct explanation by a trained team member, provision of the PIL, and an opportunity for questions, with written or electronic consent subsequently recorded. For remote participants, telephone discussions were followed by distribution of consent forms via post or email, with return options tailored to the patient's needs.

4.4.2 Launch of the Pilot Registry

Recruitment for the pilot registry commenced in January 2025. Patients are being recruited through existing clinical pathways at SJH, with recruitment facilitated via genetic counselling clinics. The process involved identifying eligible patients, obtaining informed consent, and collecting baseline data, including genetic and clinical information, to populate the registry.

4.5 Preliminary Data from the Pilot Registry

A total of 70 participants were enrolled in the preliminary Cancer Predisposing Genetic Alterations (CAmPaiGn) registry dataset at time of data reporting (29th October 2025) . All individuals provided written informed consent for inclusion. The pilot registry captured demographic, clinical, and genetic data from participants referred through both public and private healthcare systems in Ireland

4.5.1 Cohort and Consent

Of the 70 participants, 59 were female (84%) and 11 were male (16%) **(Table 4-1)**. Most participants identified as Irish (approximately 90%), with a small number of individuals of Central or Eastern European or other European origin. The majority were born in Leinster (52%), followed by Munster (21%), Connacht (14%), and Ulster (7%) **(Table 4-2)**. A small minority were born outside Ireland. The median age at first mutation diagnosis was 52 years (range 23–76). Referrals were received from a broad network of centres, most frequently St James’s Hospital (24%), Cork University Hospital (13%), University Hospital Galway (9%), and Beaumont Hospital (7%). Other sources included GPs, BreastCheck, and family referrals, demonstrating engagement from both public and private healthcare pathways.

Variable	Category	n	%
Gender	Female	59	84
	Male	11	16
Ethnicity	Irish	63	90
	Central/Eastern European	3	4
	Other European	2	3
	Other/Unknown	2	3
County of birth	Leinster	36	52
	Munster	15	21
	Connacht	10	14
	Ulster	5	7
	Outside Ireland	4	6

Median age at first mutation diagnosis		52 years	Range 23–76

Table 4-1 Demographic Characteristics of the CAmPaiGn Pilot Cohort (n = 70)

Variable	Category	n	%
Referral source	St James’s Hospital	17	24
	Cork University Hospital	9	13
	University Hospital Galway	6	9
	Beaumont Hospital	5	7
	Other hospitals and GP/family referrals	33	47
Mutation profile	Single mutation	62	89
	Dual mutation	6	9
Testing method	Panel-based	48	68
	Founder-variant testing	22	32
Testing provider	Public system	54	77
	Private system	15	21
	Self-testing	1	2
Laboratory	Blueprint Genetics	22	32
	Myriad Genetics	13	18
	West Midlands Regional Genomics Lab	10	15
	Other/unspecified	25	35
Year of testing	2023	31	44
	2024	26	37
	Other years	13	19

Table 4-2 Referral and Genetic Testing Characteristics

4.5.2 Genetic Testing and Variant Spectrum

Sixty-two participants (89%) had a single mutation identified, while six individuals (9%) carried dual mutations (**Table 4-3**). Most tests were panel-based (68%), with the remainder identified through founder-variant testing. Testing was primarily performed within the public system (77%), with additional referrals through private routes (21%) and a small proportion via self-testing. *BRCA2* was the most frequently identified pathogenic variant (41%), followed by *BRCA1* (24%). Variants were also detected in *ATM* (7%), *CHEK2* (6%), *PALB2* (4%), *MLH1* (4%), and *RAD51D* (3%). Single cases of

PMS2, *MSH6*, and *TP53* were observed. One participant carried concurrent *BRCA2* and *RAD51D* mutations. Testing facilities included Blueprint Genetics (32%), Myriad Genetics (18%), and the West Midlands Regional Genomics Laboratory (15%), with the remainder unclassified or completed through local services. Testing years were predominantly 2023 (44%) and 2024 (37%), coinciding with the national expansion of genetic testing pathways.

Gene	n	%
<i>BRCA2</i>	29	41
<i>BRCA1</i>	17	24
<i>ATM</i>	5	7
<i>CHEK2</i>	4	6
<i>PALB2</i>	3	4
<i>MLH1</i>	3	4
<i>RAD51D</i>	2	3
<i>PMS2</i>	1	1
<i>MSH6</i>	1	1
<i>TP53</i>	1	1
Other/unspecified	4	6

Table 4-3 Distribution of Pathogenic Variants

4.5.3 Disease Status and Index Cancer

At enrolment, 49 participants (70%) had a confirmed cancer diagnosis (**Table 4-4**). The most common primary cancer type was breast cancer (including bilateral cases) in 38 participants (55%), followed by ovarian cancer in 9 (13%), colorectal cancer in 6 (9%), and endometrial cancer in 4 (6%). Other malignancies included melanoma, prostate, pancreatic, and thyroid cancers, each representing fewer than 5% of total cases. The median age at first cancer diagnosis was 50 years (range 23–76). Most cancers were diagnosed between 40 and 59 years of age (64%), consistent with known patterns of hereditary cancer onset. Where disease stage was recorded, 28% were stage II, 31% were stage III, 9% were stage I, and 4% were stage IV; in 28% of cases, stage was unreported. Ten participants (14%) had a second primary cancer, most frequently contralateral breast or ovarian malignancy. Two participants had a third cancer diagnosis. Among those diagnosed through screening (approximately 22%), mammography was the predominant detection modality, followed by colonoscopy and radiological screening. Most screening occurred in high-risk contexts rather than through universal programmes.

Variable	Category	n	%
Affected status	Cancer diagnosed	49	70

	Unaffected	21	30
Primary cancer type	Breast (incl. bilateral)	38	55
	Ovarian	9	13
	Colorectal	6	9
	Endometrial	4	6
	Other (melanoma, prostate, pancreatic, thyroid, etc.)	13	17
Median age at first cancer diagnosis		50 years	Range 23–76
Second primary cancer	Yes	10	14
Third primary cancer	Yes	2	3
Stage at diagnosis	Stage I	6	9
	Stage II	20	28
	Stage III	22	31
	Stage IV	3	4
	Unknown	19	28
Mode of detection	Screening	15	22
	Symptoms/incidental	54	78

Table 4-4 Cancer-Related Characteristics

4.5.4 Family History and Cascade Testing

Family history data were available for 67 participants. Forty-four participants (66%) reported one to three affected first-degree relatives, 19 (28%) reported affected second-degree relatives, and 9 (13%) reported affected third-degree relatives (**Table 4-5**). Pathogenic variants segregating within families mirrored those observed at the individual level, with BRCA1 and BRCA2 predominating, followed by Lynch-syndrome–associated genes such as MLH1 and PMS2. Cascade testing had been initiated in 57% of families, with most reporting between one and three relatives tested. A minority of families (9%) had larger cascade testing programmes involving more than four relatives, reflecting both patient-led and clinician-supported engagement in family-based genetic testing.

Variable	Category	n	%
First-degree relatives affected	1–3	44	66
	4–6	11	16
	≥7	3	4
Second-degree relatives affected	≥1	19	28
Third-degree relatives affected	≥1	9	13
Cascade testing initiated	Yes	40	57

	No	30	43
No. of relatives tested	1–3	35	50
	4–6	6	9
	≥7	2	3

Table 4-5 Family History and Cascade Testing

4.5.5 Summary

These preliminary data confirm the feasibility of registry-based recruitment, data capture, and cross-linkage across multiple hospital systems. The dataset demonstrates broad geographic representation, successful integration of public and private testing pathways, and engagement from affected individuals and at-risk relatives. The predominance of *BRCA1* and *BRCA2* variants aligns with current national testing priorities but the inclusion of other DNA-repair genes underscores the registry’s potential to capture rare but clinically relevant genotypes.

The pilot phase validates the operational structure of the CAmPaiGn registry and establishes an essential foundation for expansion toward longitudinal outcome tracking, national cascade-testing metrics, and translational research applications such as biomarker validation through tissue arrays and collaborative linkage with larger genomic research centres.

4.6 Gathering Insights for the Registry's Development: PPI Survey

As the CAMPaIGn Study was designed as a feasibility pilot, its longer-term goal has always been to inform the development of a national registry for cancer-predisposing genetic alterations. While PPI was embedded informally throughout the pilot's development through conversations, email exchanges and event-based networking, it was recognised that a more structured and systematic approach to capturing PPI insights would be essential for national-scale planning. The study team, with the support of PPI, successfully secured funding through the Irish Association for Cancer Research (IACR) to formally advance this work. The following section outlines a PPI qualitative survey, developed and circulated as part of this next phase, aimed at capturing patient perspectives, priorities, and opinions on the design, value, and expectations of a future national registry. A PPI qualitative survey was circulated via advocacy networks using the SurveyMonkey platform. Forty-five individuals responded (100% completion rate).

4.6.1 Respondent demographics

Most respondents (95.6%, n=43) reported carrying a cancer-predisposing genetic alteration, most commonly *BRCA1/2* or Lynch syndrome, while two (4.4%) reported a strong family history without confirmed carrier status. The cohort was predominantly female (97.8%, n=44) and aged 31–60 years (95.6%, n=43). Sixteen respondents (35.6%) reported a personal history of cancer (**Table 4-6**).

Characteristic	n (%)
Gender	
Female	44 (97.8%)
Male	1 (2.2%)
Age group	
18–30	1 (2.2%)
31–45	23 (51.1%)
46–60	20 (44.4%)
Over 60	1 (2.2%)
Carrier status	
Carrier of cancer-predisposing genetic alteration	43 (95.6%)
Strong family history (no confirmed carrier)	2 (4.4%)
Healthcare professional	0 (0%)
Other	0 (0%)
Cancer diagnosis	
Yes	16 (35.6%)
No	29 (64.4%)

Table 4-6 Respondent demographics from the PPI qualitative survey**4.6.2 Engagement with the registry**

The majority (97.8%, n=44) expressed willingness to be contacted annually to update their health status, with email the preferred mode of contact (93.3%, n=42). Most participants (86.7%, n=39) indicated willingness to contribute additional health information such as new diagnoses or treatments. Nearly all respondents (93.3%, n=42) wished to receive regular updates from the registry, with email newsletters overwhelmingly preferred (95.6%, n=43) (Table 4-7).

Characteristic	n (%)
Willing to be contacted annually	44 (97.8%)
Preferred mode of contact	
- Email	42 (93.3%)
- Phone	1 (2.2%)
- Text message	4 (8.9%)
- Post	0 (0%)
Willing to contribute additional health information	39 (86.7%)
Wish to receive regular updates	42 (93.3%)
Preferred update method	
- Email newsletter	43 (95.6%)
- Online portal	2 (4.4%)
- Postal mail	0 (0%)

Table 4-7 Engagement and communication preferences**4.6.3 Data sharing preferences**

Respondents reported high levels of comfort with sharing genetic test results (93.3%, n=42), demographic data (84.4%, n=38), engagement with prophylactic surgery (88.9%, n=40), and family medical history (77.8%, n=35). A minority of respondents (n=8, 17.8%) identified categories they would prefer not to share, most frequently family history (37.5%, n=3 of 8) and demographic data (25.0%, n=2 of 8) (**Figure 4-4**).

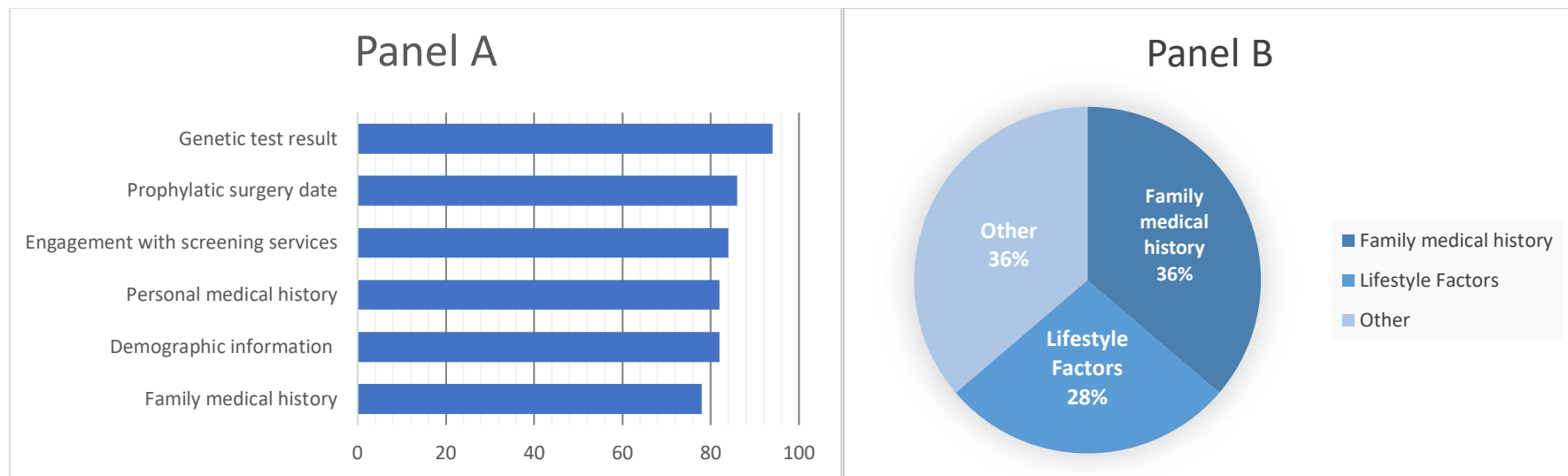


Figure 4-4 Willingness to Share Health and Genetic Data (n = 50)

Panel A shows the percentage of respondents willing to share various categories of health and genetic information with a national registry. Panel B shows the distribution of data types respondents preferred not to share.

4.6.4 Registry priorities

When asked to prioritise registry functions, monitoring outcomes such as cancer incidence and survival (82.2%, n=37) and improving access to genetic testing (75.6%, n=34) were most frequently selected. Additional priorities included facilitating clinical trial access (51.1%, n=23) and supporting research (44.4%, n=20) (**Figure 4-5**).

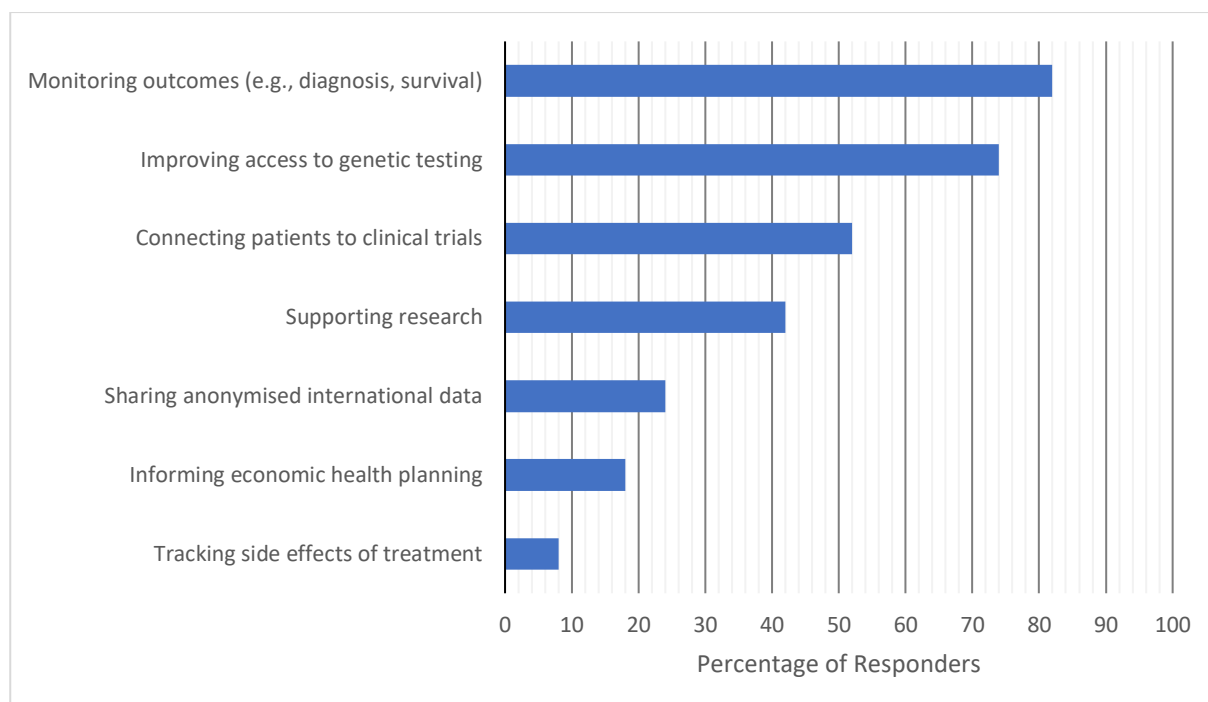


Figure 4-5 Registry Priorities and Research Linkage (n = 50)

4.6.5 Governance and oversight

Almost all respondents (97.8%, n=44) considered it very important that patients and families contribute to registry design and management. Respondents identified healthcare institutions (77.8%, n=35) and government health agencies (57.8%, n=26) as the most appropriate governing bodies, with further endorsement for advocacy organisations (44.4%, n=20) and academic institutions (35.6%, n=16) (**Table 4-8**).

Preference	n (%)
Importance of patient/family involvement in governance	
- Very important	44 (97.8%)
Preferred governing bodies	
- Healthcare institutions (e.g., hospitals, genetics clinics)	35 (77.8%)
- Government health agencies	26 (57.8%)
- Advocacy organisations / non-profits	20 (44.4%)

- Academic and research institutions	16 (35.6%)

Table 4-8 PPI governance preferences for a national registry

4.6.6 Future applications

Interest in being connected to clinical trials or research opportunities through the registry was expressed by 82.2% (n=37) of participants. Open-text responses (n=7) highlighted recurring themes of equitable access across public and private systems, transparency around data use, and the need for regular feedback to participants. Illustrative quotations included:

“It is vital that everyone in Ireland, regardless of whether they are public or private patients, has equal access to this registry.”

“Transparency in how data are used is essential if people are going to trust and contribute.”

4.7 Discussion

The development of the CAmPaiGn pilot registry represents a critical first step toward establishing a national infrastructure for the systematic collection and analysis of data on cancer-predisposing genetic alterations in Ireland. Despite Ireland's strong economic position, its eHealth infrastructure remains underdeveloped, with fragmented electronic health record (EHR) systems (340), the absence of a unified national genetic database, and complex ethical and legal considerations posing major barriers to data integration (328). In 2023, Ireland ranked lowest in the European Union for digital health integration, with only 11 percent implementation, underscoring its position as a clear outlier in healthcare digitisation (329). This context has resulted in a decentralised and disjointed data landscape(340), where existing genetic information remains dispersed across hospitals, diagnostic laboratories, and research databases, with no national infrastructure linking these data to clinical outcomes (95). Although the National Cancer Registry Ireland (NCRI) provides comprehensive information on cancer incidence and survival (83), it does not currently collect or integrate genetic data (94). Consequently, the prevalence and clinical impact of cancer-predisposing alterations, such as *BRCA1/2* and Lynch syndrome, within the Irish population remain poorly characterised.

These limitations were directly addressed through the design and implementation of the CAmPaiGn feasibility pilot. The pilot identified key logistical and governance challenges including fragmented health record systems and variable institutional data protection practices which were mitigated through structured stakeholder engagement, inter-institutional collaboration, and the development of formalised governance procedures. Importantly, the pilot registry embedded PPI from the outset, ensuring that its design, structure, and oversight were informed by individuals with lived experience of hereditary cancer syndromes (341-343). This participatory approach aligns with international best practice, fostering trust and transparency in the collection and use of genomic data.

Complementing the pilot, a national survey of individuals with cancer-predisposing genetic alterations demonstrated strong public support for the establishment of such a registry. Respondents expressed willingness to share health and genetic information, engage in longitudinal data collection, and contribute to research or clinical initiatives, provided that data security and governance were assured. The consistency of this support across age groups and clinical backgrounds reflects a collective understanding of the potential societal benefit of a coordinated approach to hereditary cancer risk data in Ireland. Over 90 percent of respondents reported comfort sharing sensitive genetic information, highlighting both trust in publicly governed health institutions and the feasibility of a national, multi-domain registry model. Respondents preferred digital engagement, with most favouring email communication for registry contact and updates. This preference aligns with ongoing digital transformation efforts within the Health Service Executive (HSE)(344).

The CAmPaiGn registry was developed in direct response to this readiness. It provides a framework

for harmonising demographic, clinical, and genetic data to support clinical care and translational research. While its immediate role is to consolidate foundational information on Irish individuals with hereditary cancer predisposition, its long-term potential lies in enabling integration of diagnostic and prognostic biomarkers to advance precision oncology (20). Over time, the dataset could support computational and bioinformatic models capable of analysing large-scale genomic information to identify clinically actionable patterns. Such *in silico* approaches, when combined with translational and clinical data, could facilitate prediction of treatment response and guide development of novel therapeutic strategies.

Lessons from established international models including the National Inherited Cancer Predisposition Register (NICPR) in England (345), the Dutch Hereditary Cancer Registry (346) and the Danish HNPCC Register (346) illustrate how coordinated national data collection can improve early detection, treatment, and survival outcomes (347). Unlike most of these countries, Ireland lacks both a national electronic identifier and a centralised health data repository. However, lessons from smaller European countries such as Slovenia, successfully implemented a hereditary cancer registry through *mandated reporting* of results from genetic testing providers (347), may therefore be a particularly relevant model. Within Ireland, the Cystic Fibrosis Registry provides a strong precedent, demonstrating that a registry can be successfully established through independent, privately funded efforts before transitioning to public funding and national integration (348).

Ultimately, the success of a national hereditary cancer registry will depend on sustained investment, interoperability between systems, and continued public trust. The CAMpaiGn pilot provides a tested framework for addressing these challenges. By embedding governance, data protection, and PPI principles from inception, it establishes a scalable model for national implementation. Its development represents a pivotal step toward building a cohesive genomic ecosystem capable of linking clinical, molecular, and patient-reported data to improve care, enable precision prevention, and strengthen outcomes for individuals and families affected by inherited cancer risk.

Expression Profiling of miRNA in Ovarian Cancer

Chapter Five

5 Expression Profiling of miRNA in Ovarian Cancer

5.1 Introduction

HGSOC has a five-year survival rate of less than 45% (20), highlighting the urgent need for novel prognostic markers to enhance personalized treatment strategies. miRNAs, small non-coding RNAs that regulate approximately 30% of human genes (114), play significant roles in various biological processes, including cell proliferation and apoptosis. However, inconsistencies in miRNA sensitivity and specificity for identifying OC complicate their potential clinical application (121). Conflicting findings regarding miRNA expression often arise from factors such as sample size and tumour heterogeneity (122).

In this context, *in silico* approaches have emerged as valuable tools for addressing these challenges (349). By leveraging computational methods, researchers can analyse large-scale genomic and transcriptomic datasets, allowing for the identification of potential miRNA-target gene relationships and the elucidation of intricate signalling pathways (350). *In silico* analyses can help integrate diverse data sources, including clinical outcomes and molecular profiles, revealing patterns and correlations that may not be apparent in traditional experimental studies (351). For instance, bioinformatics tools can facilitate the discovery of miRNA signatures associated with specific OC subtypes or stages, thus providing insights into disease progression and potential therapeutic targets (131).

Moreover, *in silico* modelling can simulate the effects of miRNA dysregulation on tumour behaviour, helping to predict how these molecules might influence processes like epithelial-to-mesenchymal transition (EMT) (168), an important process which facilitates tissue remodelling during embryonic development and is viewed as an essential early step in tumour metastasis (352). This computational approach not only enhances our understanding of the complex interactions within cancer biology but also accelerates the validation of miRNA candidates as biomarkers by streamlining the hypothesis generation process.

Despite the difficulties in validating miRNAs as clinical biomarkers, their presence in tumour tissues and body fluids makes them attractive candidates (353). Recent advancements in detection technologies, including miRNA microarrays and high-throughput sequencing, have improved our understanding of their roles in cancer (350). Some miRNAs function as tumour suppressors by downregulating oncogenes, while others promote tumour growth and metastasis by targeting tumour suppressor genes and disrupting signalling pathways.

The intricate interplay of miRNAs in OC highlights their potential as biomarkers and therapeutic targets (125, 354). Ongoing research, especially through *in silico* methods, is crucial for elucidating the molecular mechanisms underlying OC and translating these insights into effective clinical strategies (349). While *in silico* analyses offer valuable perspectives, it is imperative that these findings are validated through *in vivo* studies to ensure their clinical relevance. Bridging

computational and experimental approaches will be essential for advancing our understanding of OC and developing impactful treatment strategies to enhance patient outcomes.

5.2 Aims and Objectives

5.2.1 General Aims

The general aim of this chapter is to examine the expression profile of miRNA in OC cell lines.

Through these objectives, the aim is to enhance understanding of the molecular mechanisms underlying OC pathogenesis and potentially identify novel avenues for prognosis, diagnosis and treatment.

5.2.2 Hypothesis

The hypothesis underpinning this study is that specific miRNA expression profiles significantly influence the development and progression of OC, with variations observed based on *BRCA* status.

5.2.3 Objectives

The objectives of this chapter are

- Identify differential expression patterns of miRNAs associated with OC cell lines across different *BRCA* status.
- Validate selected miRNAs in these cell lines.

5.3 Results

5.3.1 miRNA Identified from In Silico Analysis of OC

To identify the miRNAs present in the three selected OC cell lines (OVSAHO, COV362, and KURAMOCHI), differences in expression of the miRNA unique to each cell line and the miRNA shared among them were assessed using the NEXTFLEX Rapid Directional RNA-Seq Kit 2.0 (PerkinElmer) as outlined in **Section 2.2.2**. The miRNAs of interest were categorized into four groups (labelled 1 – 4, Figure 4.1) and selected as visualized in a Venn diagram below.

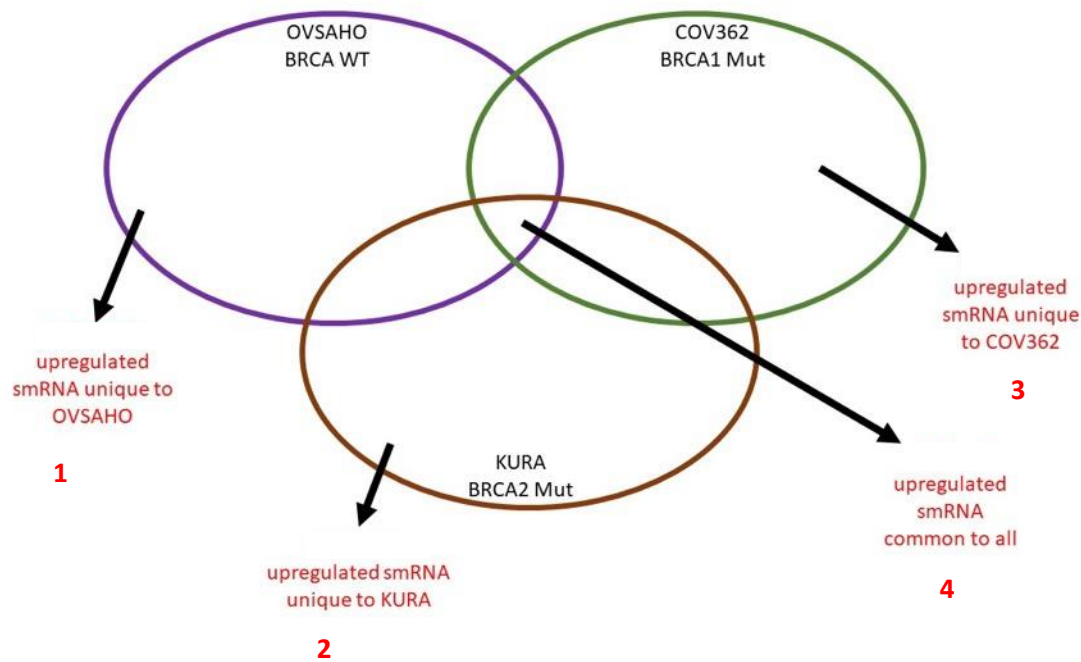


Figure 5-1 Venn diagram illustrating the selection conditions of miRNAs

The results revealed normalized miRNA expression levels, with fold changes calculated for the following conditions. Condition 1: miRNAs unique to OVSAHO, Condition 2: miRNAs unique to KURAMOCHI, Condition 3: miRNAs unique to COV362, and Condition 4: miRNAs common to all three cell lines. For instance, miRNAs identified as unique to OVSAHO were specifically upregulated in OVSAHO, excluding those also upregulated in COV362 and KURAMOCHI. The normalized miRNAs were ranked according to their fold change values. In total, 44 miRNAs of interest were identified. The results for each miRNA category are presented in **Table 5-1**.

miRNA unique to OVSAHO		miRNA unique to KURA		miRNA unique to COV362		miRNA common to all cell lines	
rank	miRNA	rank	miRNA	rank	miRNA	rank	miRNA
1	hsa-let-7a-3p	1	hsa-miR-27b-3p	1	hsa-miR-376c-3p	1	hsa-miR-30c-5p
2	hsa-miR-590-3p	2	hsa-miR-23a-3p	2	hsa-miR-3176	2	hsa-miR-26a-5p
		3	hsa-let-7f-5p	3	hsa-miR-6820-3p	3	hsa-miR-143-3p
		4	hsa-miR-27a-3p	4	hsa-miR-5700	4	hsa-miR-15b-5p
		5	hsa-miR-335-5p	5	hsa-miR-4484	5	hsa-miR-423-3p
		6	hsa-let-7g-5p			6	hsa-miR-93-5p
		7	hsa-let-7b-5p			7	hsa-miR-3613-5p
		8	hsa-let-7i-5p			8	hsa-miR-101-3p
		9	hsa-miR-135a-5p			9	hsa-miR-339-5p
		10	hsa-miR-335-3p			10	hsa-miR-126-3p
		11	hsa-miR-10a-5p			11	hsa-miR-30a-3p
		12	hsa-miR-106b-3p			12	hsa-miR-145-5p
		13	hsa-miR-98-5p			13	hsa-miR-378a-3p
		14	hsa-miR-28-5p			14	hsa-miR-652-3p
		15	hsa-miR-205-5p			15	hsa-miR-4485-3p
		16	hsa-miR-30e-3p			16	hsa-miR-606
		17	hsa-miR-574-3p			17	hsa-miR-4296
		18	hsa-miR-193b-			18	hsa-miR-4330

			3p				
						19	hsa-miR-196b-5p

Table 5-1 Upregulated miRNA categories of interest across cell lines

5.3.2 smRNA In Silico assessment

5.3.2.1 KMplotter Prediction Analysis

To assess the prognostic value of key differentially expressed miRNAs identified as upregulated through the GFOLD algorithm, the web-based tool KMplotter (257) was utilized to perform comprehensive survival analysis and calculate quantitative measures for each miRNA. The expression levels of the selected miRNAs in OC patients were analyzed for statistically significant prognostic value (see Figure 4-2). MiRNAs that had an FDR $\neq$ 100%, were not flagged for low median expression, and had p-values < 0.05 (Figure 4-2) were selected for further evaluation. This resulted in the identification of 11 miRNAs for deeper analysis. These miRNAs were then assessed using the KMplotter Prediction Analysis Tool to determine whether high or low expression levels were associated with a statistically significant difference in survival. The hazard ratios (HR) for overall survival (OS), p-values, and median survival times (in months) for high and low expression levels of these 11 miRNAs are detailed in Table 4-2.

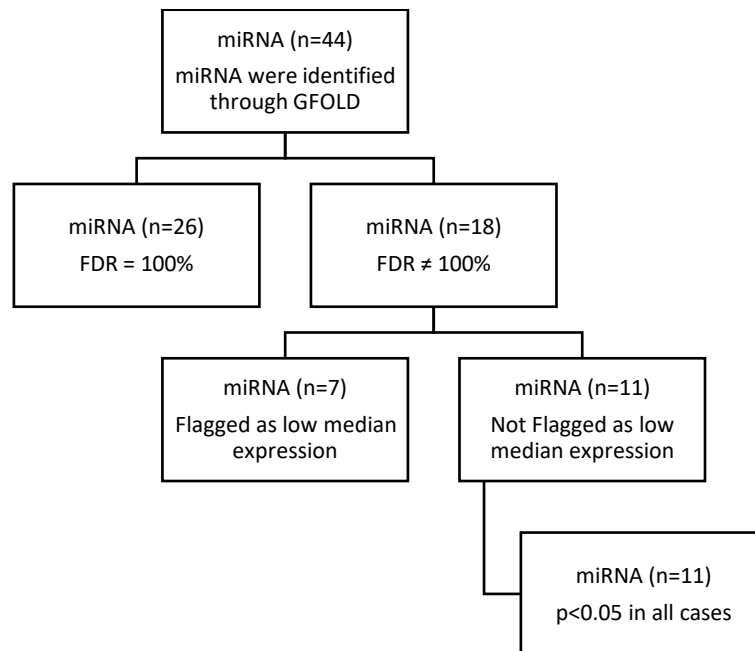


Figure 5-2 Consort Diagram showing selection process from miRNA in KMPlotter

Upregulated miRNA Cell Line	miRNA	HR for OS (months)	p value	Median Survival Low Expression	Median Survival High Expression	Difference in Survival
OVSAHO	hsa-let-7a-3p	1.29 (1 - 1.67)	*	45.77	45.13	0.64
OVSAHO	hsa-miR-590-3p	0.63 (0.49 - 0.83)	**	42.63	56.7	-14.07
KURAMOCHI	hsa-miR-27b-3p	1.35 (1.07 - 1.69)	*	48.07	43.8	4.27
KURAMOCHI	hsa-let-7f-5p	0.73 (0.57 - 0.93)	*	39.97	48.2	-8.23
KURAMOCHI	hsa-miR-10a-5p	1.31 (1.04 - 1.65)	*	48.37	41.63	6.74
KURAMOCHI	hsa-miR-98-5p	0.73 (0.58 - 0.92)	**	41.63	52.77	-11.14
KURAMOCHI	hsa-miR-205-5p	0.71 (0.56 - 0.91)	**	40.97	52.63	-11.66
COV362	hsa-miR-3176	1.4 (1.1 - 1.78)	**	51.73	41.97	9.76
Common to all	hsa-miR-3613-5p	1.27 (1.01 - 1.6)	*	46.63	42.2	4.43
Common to all	hsa-miR-652-3p	0.68 (0.54 - 0.85)	**	41.97	49.43	-7.46
Common to all	hsa-miR-196b-5p	1.33 (1.06 - 1.66)	*	50.3	41.63	8.67

Table 5-2 OS for selected miRNA from KM Plotter¹

¹ Statistical significance is indicated as follows, as per 2024 GraphPad Software: Nos (not significant, $P > 0.05$), * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), and **** ($P \leq 0.0001$)

5.3.2.2 Target Scan Prediction Analysis

The selected miRNAs underwent comprehensive prediction analysis using TargetScan (258).

TargetScan employs advanced algorithms to predict miRNA binding sites within mRNA sequences, facilitating the identification of potential miRNA-gene regulatory interactions. After entering the selected miRNA gene signatures into the online tool, the results were filtered specifically for human species, producing a comprehensive table of target genes associated with each miRNA (**Table 5-3**).

Among the analysed miRNAs, let-7f-5p and miR-98-5p ((highlighted in bold below) were identified as analogous, highlighting their potential relevance in gene regulation.

miRNA	Top1 Gene	Top2 Gene	Top3 Gene	Top4 Gene	Top5 Gene
let-7a-3p	ZNF460	CCL7	POLR2K	TAS2R14	RP11-386G21.1
miR-590-3p	ZNF804B	NECAB1	INO80D	DUSP16	ZNF253
miR-27b-3p	GCC2	PLK2	AKIRIN1	NOVA1	SZRD1
let-7f-5p	HMGA2	ARID3B	LIN28B	FIGN	TRIM71
miR-10a-5p	BDNF	ARSJ	CRLF3	TFAP2C	HOXA3
miR-98-5p	HMGA2	ARID3B	LIN28B	FIGN	TRIM71
miR-205-5p	BICC1	TNFAIP8	CHN1	NEU1	C11orf86
miR-3176	C17orf62	PEX10	TLCD1	SLC48A1	CST3
miR-3613-5p	RRAGD	ZNF791	CADM2	ZNF83	ANKRD45
miR-652-3p	ISL1	TNRC6A	NPTN	CAPZB	PRRX1
miR-196b-5p	HOXC8	HOXB8	HOXA7	NR6A1	RP1-170O19.20

Table 5-3 Genes Associated with selected miRNA as evaluated in TargetScan

5.3.2.3 UALCAN TCGA Dataset Analysis

The genes identified as being associated with the miRNA, where then interrogated in the UALCAN TCGA Dataset. Three genes (*RP11-386G21.1*, *RP1-170O19.20* and *SZRD1*) did not have information available. N/A indicates that there was not sufficient information available.

Expression of these genes according to stages (UALCAN) is summarized in **Table 5-4**, and statistical significance reviewed in UALCAN is shown by stage. Of note, only BICC was statistically significant for survival with a p-value of * The non-significant genes identified in the UALCAN TCGA Dataset are presented in **Appendix 4** for reference.

GENE RANK to miRNA	Gene	Expression on Survival	Expression of gene according to stages (UALCAN), statistical significance		
		p Value	Stage2 vs Stage3	Stage2 vs Stage4	Stage3 vs Stage4
2	<i>ARSJ</i>	Nos	*	Nos	Nos
1	<i>BICC1</i>	*	Nos	Nos	Nos
3	<i>CADM2</i>	Nos	*	Nos	***
4	<i>DUSP16</i>	Nos	Nos	*	*
2	<i>HOXB8</i>	Nos	*	*	Nos
3	<i>INO80D</i>	Nos	*	*	Nos
1	<i>ISL1</i>	Nos	*	Nos	Nos
3	<i>LIN28B</i>	Nos	Nos	Nos	*
4	<i>NR6A1</i>	Nos	Nos	Nos	*
2	<i>PEX10</i>	Nos	**	Nos	Nos
3	<i>POLR2K</i>	Nos	*	Nos	Nos
5	<i>PRRX1</i>	Nos	***	*	Nos
4	<i>TAS2R14</i>	Nos	**	**	Nos
2	<i>TNRC6A</i>	Nos	**	*	Nos
1	<i>ZNF460</i>	Nos	*	Nos	Nos
1	<i>ZNF804B</i>	Nos	Nos	Nos	**

Table 5-4 Association of gene expression with tumour stage and survival analysed in UALCAN.

Expression levels of 15 genes identified through miRNA target ranking were assessed for associations with survival and tumour stage in ovarian cancer using the UALCAN database. Statistical significance is indicated as follows: Nos (not significant, $p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), and **** ($p \leq 0.0001$), as per 2024 GraphPad Software.

5.3.2.4 UALCAN Proteomic Expression Profile

The genes associated with the miRNA were further analysed using the UALCAN Proteomic Expression Profile tool. Among the 50 genes examined, information was available for 24. Among these, 12 genes exhibited statistical significance ($p < 0.05$) when comparing protein expression between normal tissue and tumour tissue (**Table 5-5**). Within these 12 genes, 11 displayed statistical significance in protein expression differences between normal tissue and grade 3 tumours, as well as between normal tissue and stage 3 or stage 4 tumours, where the sample distribution was as follows: normal tissue (n=25), Stage 1 (n=2), Stage 3 (n=75), and Stage 4 (n=16). N/A indicates that there was not sufficient information available. The non-significant genes identified are presented in **Appendix 5**.

GENE RANK to miRNA	Gene	Comparison Normal V All Samples	Comparison Normal V Grade 3	Comparison Normal V Stage 4	Comparison Normal V Stage 3
5	BICC1	**	*	n/a	Nos
3	CRLF3	****	****	Nos	****
3	DUSP16	Nos	****	n/a	Nos
1	GCC2	****	****	Nos	***
2	HMG2	**	*	****	**
1	INO80D	Nos	Nos	N/A	*
1	NEU1	Nos	Nos	*	Nos
5	NPTN	****	***	Nos	***
1	POLR2K	****	****	Nos	****
3	PRRX1	****	****	N/A	****
5	SZRD1	***	***	Nos	*
4	TNFAIP8	**	**	Nos	***
5	TNRC6A	Nos	Nos	N/A	Nos
2	ZNF253	****	****	Nos	****
3	ZNF460	****	****	Nos	****
4	ZNF791	****	****	Nos	****
4	ZNF804B	Nos	Nos	N/A	**

Table 5-5 Statistical significance of gene related protein expression between normal and OC.

Protein expression levels of 17 genes, identified through miRNA target ranking, were analysed using the UALCAN database to compare normal ovarian tissue and OC samples across clinical subgroups. Comparisons were performed between normal samples and all OC samples, as well as between normal and advanced disease stages (Grade 3, Stage 3, and Stage 4). Statistical significance is indicated as follows: Nos (not significant, $p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), and **** ($p \leq 0.0001$), as per 2024 GraphPad Software.

Therefore the 12 most significant genes are outlined in **Table 5-6**, and the related cell line and *BRCA* status outlined.

Gene	GENE RANK to miRNA	Related miRNA	Related Cell Line	Related BRCA Status
ZNF791	2	hsa-miR-652-3p	Common	n/a
PRRX1	5	hsa-miR-196b-5p	Common	n/a
BICC1	1	hsa-miR-3176	Unique to COV362	<i>BRCA1</i> Mutant
TNFAIP8	4	hsa-miR-3176	Unique to COV362	<i>BRCA1</i> Mutant
GCC2	1	hsa-miR-27b-3p	Unique to KURAMOCHI	<i>BRCA2</i> Mutant
HMGA2	1	hsa-miR-205-5p	Unique to KURAMOCHI	<i>BRCA2</i> Mutant
CRLF3	3	hsa-miR-98-5p	Unique to KURAMOCHI	<i>BRCA2</i> Mutant
NPTN	3	hsa-miR-205-5p	Unique to KURAMOCHI	<i>BRCA2</i> Mutant
SZRD1	5	hsa-miR-27b-3p	Unique to KURAMOCHI	<i>BRCA2</i> Mutant
ZNF460	1	hsa-let-7a-3p	Unique to OVSAHO	<i>BRCA</i> Wildtype
POLR2K	3	hsa-let-7a-3p	Unique to OVSAHO	<i>BRCA</i> Wildtype
ZNF253	5	hsa-miR-590-3p	Unique to OVSAHO	<i>BRCA</i> Wildtype

Table 5-6 Summary of the most significant identified genes, related cell line and BRCA status

5.3.3 Comparison between findings and current literature

By compiling the miRNA-associated genes identified in TargetScan (**Table 5-5**) a PubMed search was conducted to assess if the genes identified above were previously associated with OC (**Table 5-7**). The time of literature retrieval was up to March 2024. Details of the search strings utilized can be found in **Appendix 6**. The search strategy involved querying PubMed for OC studies associated with the genes identified. Initially, a search of the full set of genes yielded 149 results over a 22-year period (2002–2024). The search was then refined to include only the significant genes listed in (**Table 5-6**), resulting in 54 articles, with the majority focusing on HMGA2. To further narrow the results, HMGA2 was excluded, which reduced the article count to 11.

GENE	Title	Year	Author
TNFAIP8	TIPE1 (TNFAIP8) Suppresses Growth and Metastasis of Ovarian Cancer	2021	Zhang et al. (355)
	TNFAIP8 variants as potential epidemiological and predictive biomarkers in ovarian cancer	2020	Gao et al. (356)
	TNFAIP8 promotes cell growth by regulating the Hippo pathway in epithelial ovarian cancer	2018	Xie et al. (357)
	TNFAIP8 overexpression is associated with platinum resistance in epithelial ovarian cancers with optimal cytoreduction	2014	Liu et al. (358)
	TNFAIP8 as a predictor of metastasis and a novel prognostic biomarker in patients with epithelial ovarian cancer	2013	Liu et al. (359)
	Tumor necrosis factor α -induced protein 8 (TNFAIP8) expression as a predictor of prognosis and resistance in patients with advanced ovarian cancer treated with neoadjuvant chemotherapy	2018	Wang et al. (360)
BICC1	TRIM29 regulates the SETBP1/SET/PP2A axis via transcription factor VEZF1 to promote progression of ovarian cancer	2022	Qiao et al. (361)
PRRX1	Activation of COL11A1 by PRRX1 promotes tumor progression and radioresistance in ovarian cancer	2021	Zhu et al. (362)
	Prospective pathway signaling and prognostic values of MicroRNA-9 in ovarian cancer based on gene expression omnibus (GEO): a bioinformatics analysis	2021	Zuo et al. (363)

	Expression and prognostic significance of epithelial-mesenchymal transition-related markers and phenotype in serous ovarian cancer	2018	In Hye et al. (364)
ZNF253	The plasma peptides of ovarian cancer	2018	Dufresne et al. (365)

Table 5-7 miRNA-associated genes literature review

5.3.4 Prognostication of genes as reviewed in cBioportal

Selected genes were analyzed using the cBioPortal platform, a comprehensive cancer genomics resource, to explore their potential impact on patient survival. By integrating the genetic alterations with clinical data, the platform was used to generate gene-associated survival outcomes.

(vii)HMGA2

According to data from cBioportal, HMGA2 is a gene that undergoes alterations in 3.6% of Ovarian Serous Cystadenocarcinoma cases (21 out of 584 samples). The median OS for patients without any alterations in the HMGA2 gene is 66.74 months (95% CI 64.44 - 70.82), while it decreases to only 39.89 months (95% CI 35.77 - 60.00) for those with altered HMGA2. This is demonstrated in the Kaplan-Meier curve shown in **Figure 5-3**. This difference is statistically significant ($p = 0.0002827$).

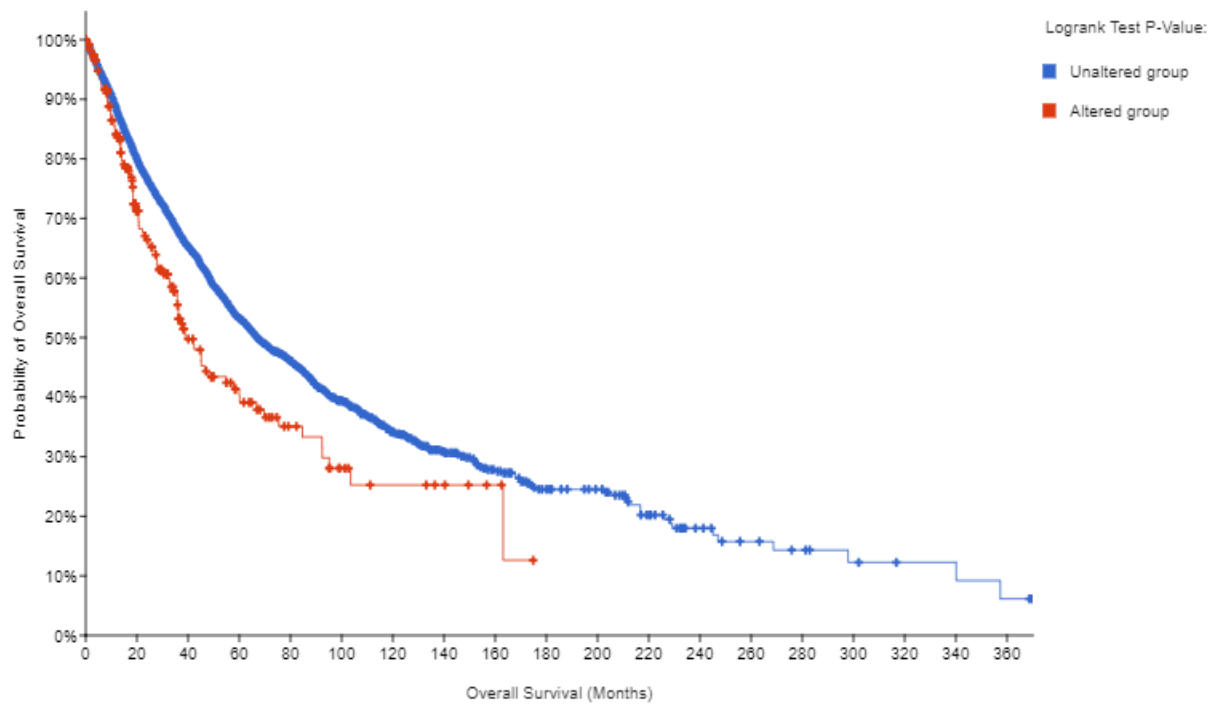


Figure 5-3 Survival associated with HMLA2 in OC

(viii) **TNFAIP8**

According to data from cBioportal, TNFAIP8 is a gene that undergoes alterations in 3.08% of Ovarian Serous Cystadenocarcinoma cases. These alterations include mutation (0.17%), amplification (1.54%) and deep deletion (0.37%). The median OS months decreases from 66.57 (95% CI 63.98 - 70.16) months in the unaltered group to only 55.76 (95% CI 47.54 - NA) months in the altered groups, however this difference was not statistically significant ($p = 0.349$) (**Figure 5-4**).

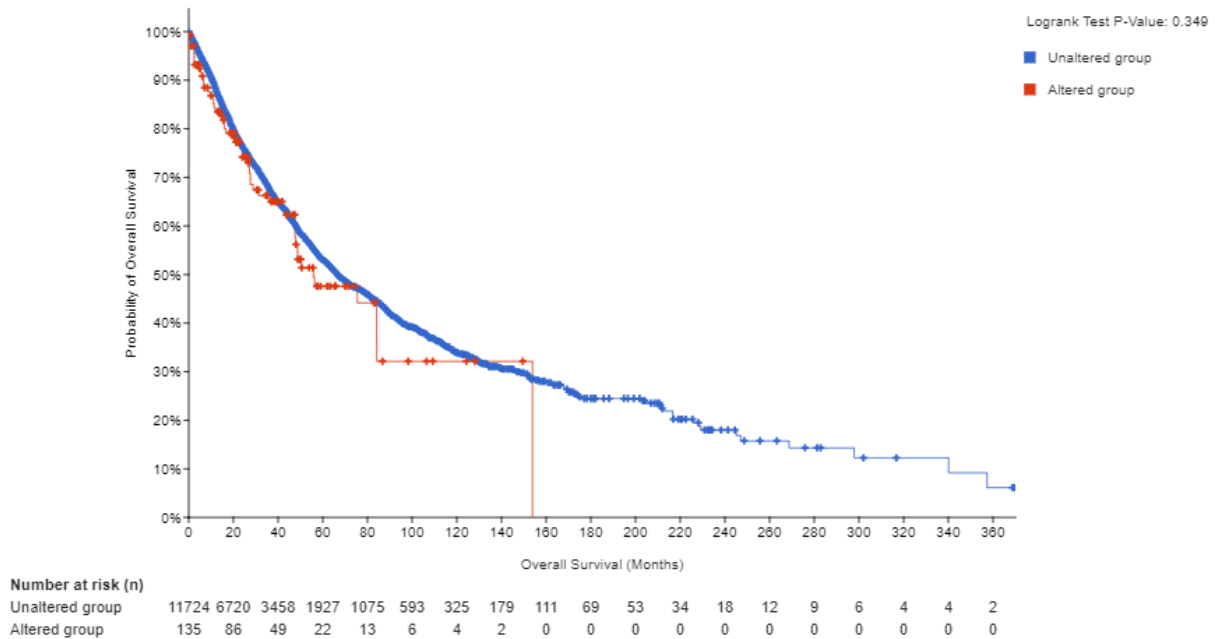


Figure 5-4 Survival associated with TNFAIP8 in OC

(ix) BICC1

According to data from cBioportal, BICC1 is a gene that undergoes alterations 2.4% of Ovarian Serous Cystadenocarcinoma cases (12 out of 584 samples). These alterations include mutation (0.51%) and amplification (1.88%). The median OS months decreases from 66.41 (95% CI 63.93 - 69.93) months in the unaltered group to only 84.13 (95% CI 61.66 - 112.57) months in the altered groups, however this difference was not statistically significant ($p=0.171$) (**Figure 5-5**).

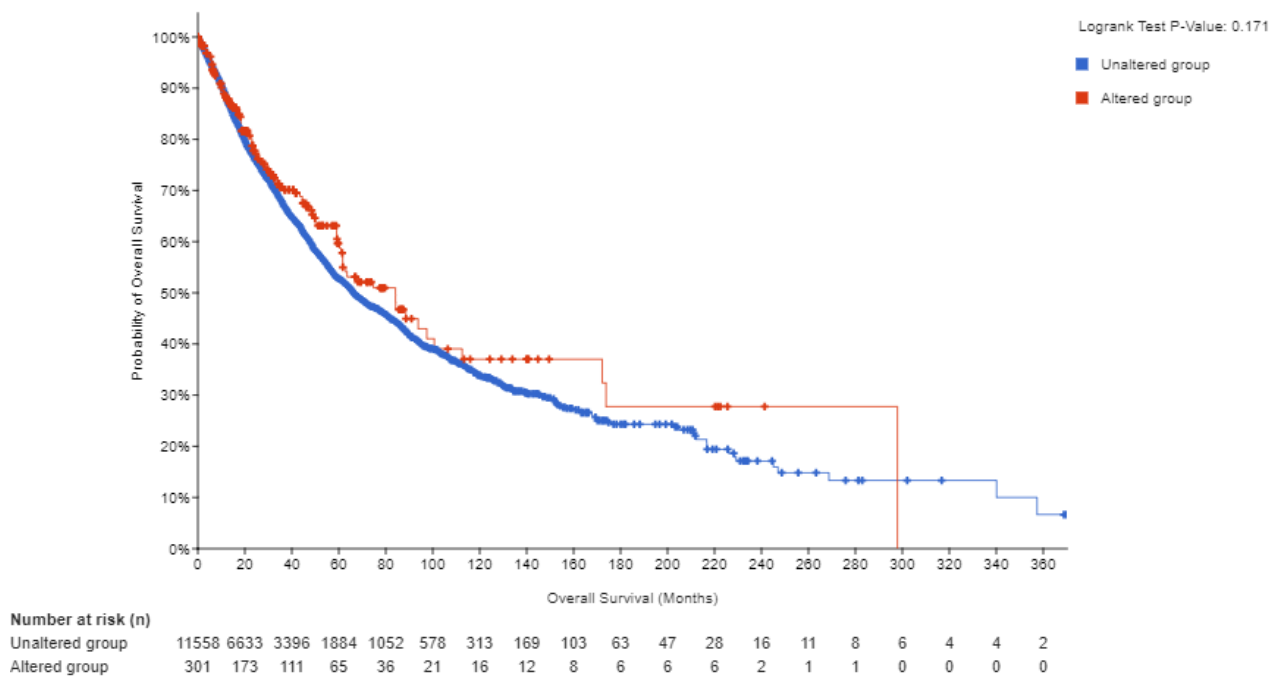


Figure 5-5 Survival associated with BICC1 in OC

(x) PRRX1

According to data from cBioportal, HMGA2 is a gene that undergoes alterations in 4.79% of Ovarian Serous Cystadenocarcinoma cases (28 out of 584 samples). These alterations include mutation (0.17%) and amplification (4.62%). The median OS months (95% CI) decreases from 66.41 (95% CI 63.93 - 69.90) months in the unaltered group to only 75.48 (95% CI 55.39 - 100.70) months in the altered groups ($p=0.74$) (**Figure 5-6**).

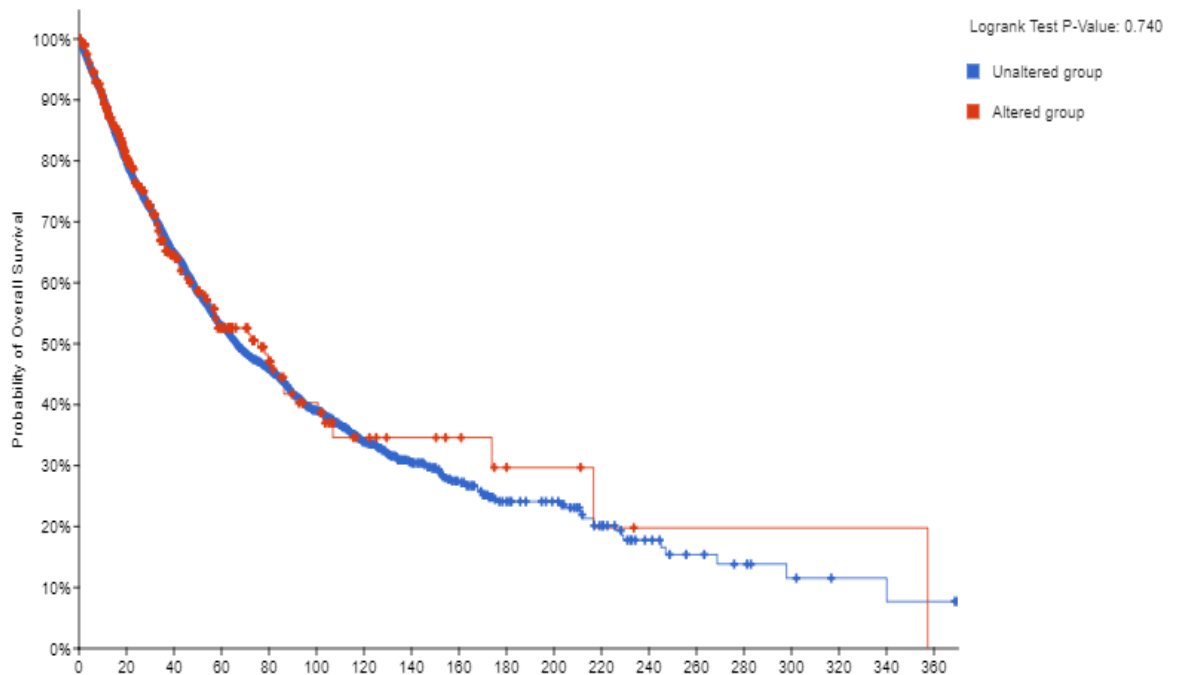


Figure 5-6 Survival associated with PRRX1 in OC

5.4 In Vitro Validation of selected miRNA

5.4.1 Selection of miRNA Targets and primer design

miRNA targets were selected based on in silico analysis as described. Following this selection process, miRNA primers were designed to facilitate subsequent experimental validation. The twelve most significant genes identified in this analysis are detailed in **Table 5-8**, which also includes information on the related cell lines used in the study and their respective *BRCA* status.

Gene	GENE RANK to miRNA	Related miRNA	Related Cell Line	Related BRCA Status
ZNF791	2	hsa-miR-652-3p	Common	n/a
PRRX1	5	hsa-miR-196b-5p	Common	n/a
BICC1	1	hsa-miR-3176	Unique to COV362	<i>BRCA1</i> Mutant
TNFAIP8	4	hsa-miR-3176	Unique to COV362	<i>BRCA1</i> Mutant
GCC2	1	hsa-miR-27b-3p	Unique to KURAMOCHI	<i>BRCA2</i> Mutant
HMGA2	1	hsa-miR-205-5p	Unique to KURAMOCHI	<i>BRCA2</i> Mutant
CRLF3	3	hsa-miR-98-5p	Unique to KURAMOCHI	<i>BRCA2</i> Mutant
NPTN	3	hsa-miR-205-5p	Unique to KURAMOCHI	<i>BRCA2</i> Mutant
SZRD1	5	hsa-miR-27b-3p	Unique to KURAMOCHI	<i>BRCA2</i> Mutant
ZNF460	1	hsa-let-7a-3p	Unique to OVSAHO	<i>BRCA</i> Wildtype
POLR2K	3	hsa-let-7a-3p	Unique to OVSAHO	<i>BRCA</i> Wildtype
ZNF253	5	hsa-miR-590-3p	Unique to OVSAHO	<i>BRCA</i> Wildtype

Table 5-8 Summary of genes comparing protein expression between normal and tumour tissue

5.4.2 Optimisation of primers

Primers were optimized across three distinct cell line - COV362, KURAMOCHI, and OVSAHO - utilizing endpoint PCR techniques to ensure effective amplification of the target sequences. The optimization process involved systematic adjustments to various parameters, including primer concentration, annealing temperature, and cycling conditions, to achieve optimal specificity and efficiency of the primers. Once the PCR was completed, the resulting products were analyzed through agarose gel electrophoresis. This step allowed for the visualization of the PCR products, enabling assessment of both primer specificity, which ensures that only the desired target sequences were amplified, and primer efficiency, which quantifies the effectiveness of the amplification process. Detailed methodologies and specific conditions employed during this optimization process are outlined in section 2.2.4, providing a comprehensive overview for reproducibility and further experimental planning. Figures 4-7 to 4-21 present the gel images of the optimized endpoint PCR for the selected

primers. Upon evaluation, some primers were deemed to exhibit superior quality in terms of band intensity and specificity compared to others.

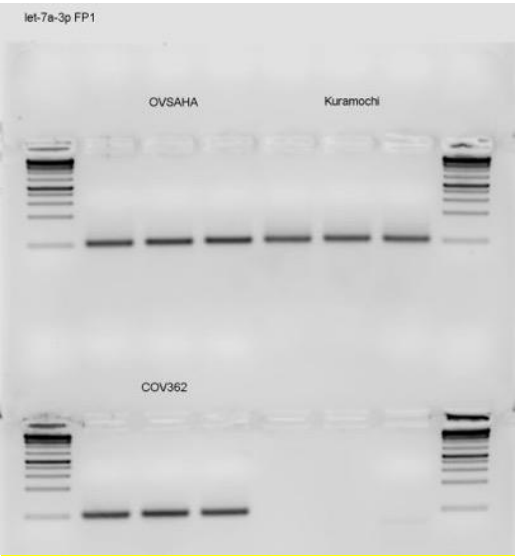


Figure 5-7 miRNA let-7a-3p Forward Primer 1

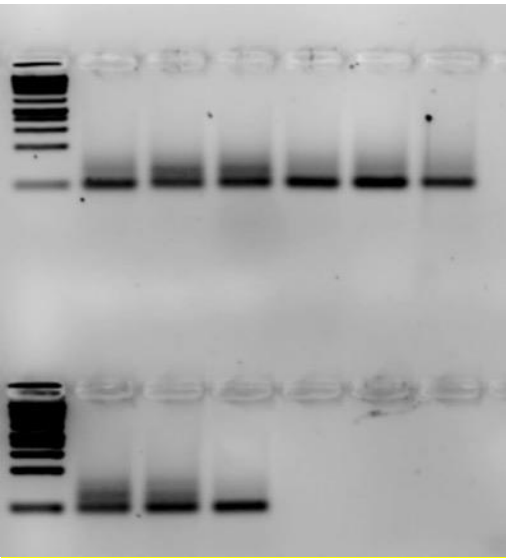


Figure 5-8 hsa-miR-590-3p Forward Primer 1

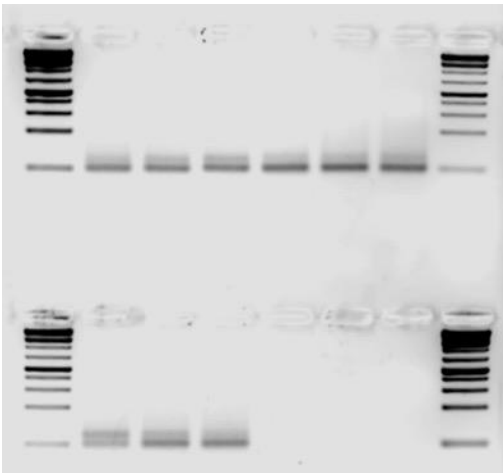


Figure 5-9 hsa-miR-590-3p Forward Primer 2

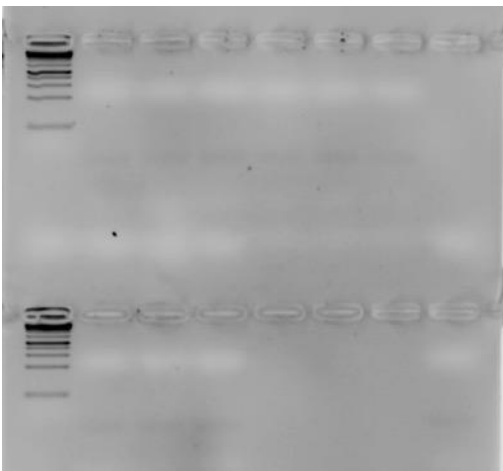


Figure 5-10 hsa-miR-27b-3p Forward Primer 1

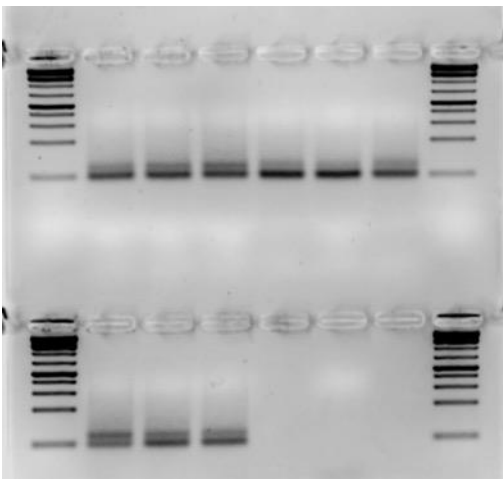


Figure 5-11 hsa-miR-27b-3p Forward Primer 2

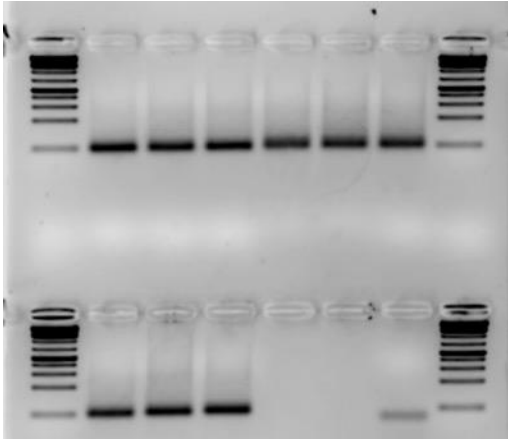


Figure 5-12 hsa-let-7f-5p Forward Primer 1

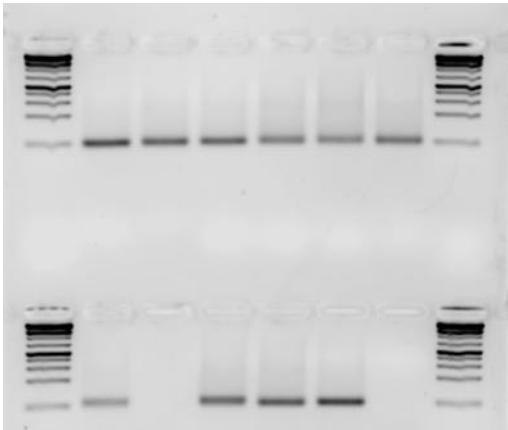


Figure 5-13 hsa-miR-98-5p Forward Primer 1

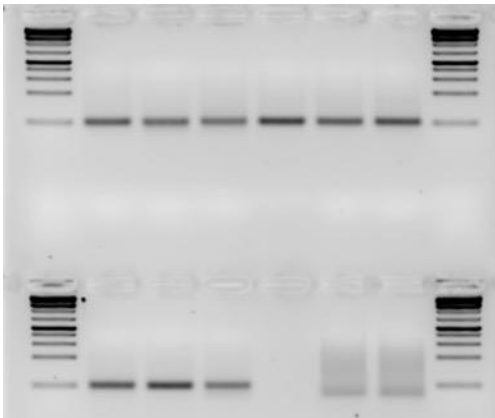


Figure 5-14 hsa-miR-205-5p Forward Primer 1

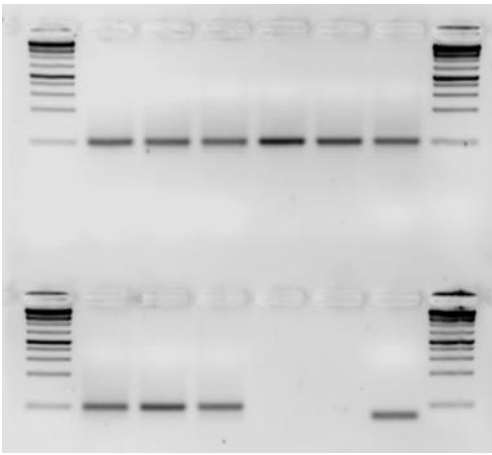


Figure 5-15 hsa-miR-205-5p Forward Primer 2

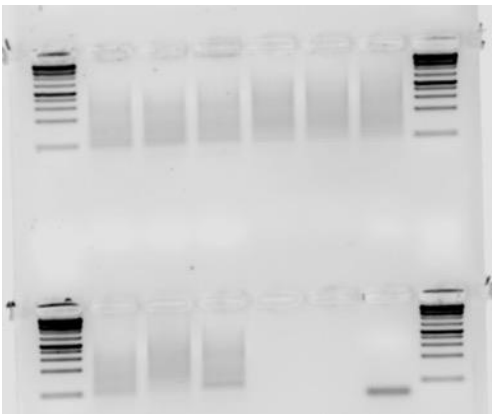


Figure 5-16 hsa-miR-3176 FP1

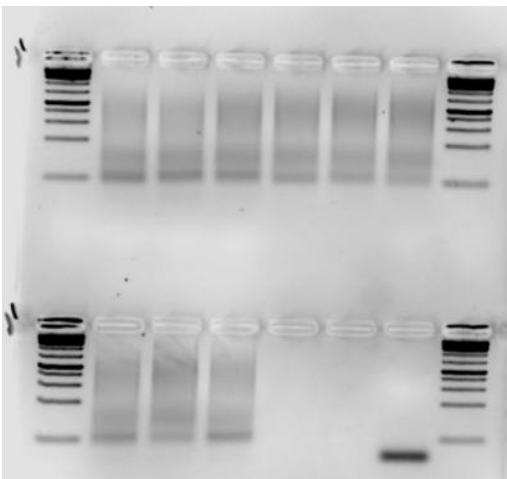


Figure 5-17 hsa-miR-3176 FP2

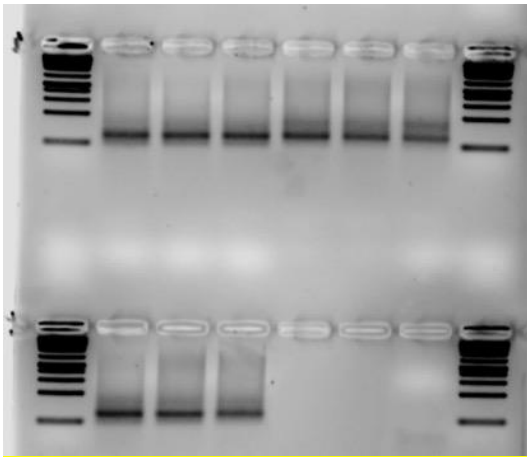


Figure 5-18 hsa-miR-3613-5p FP1

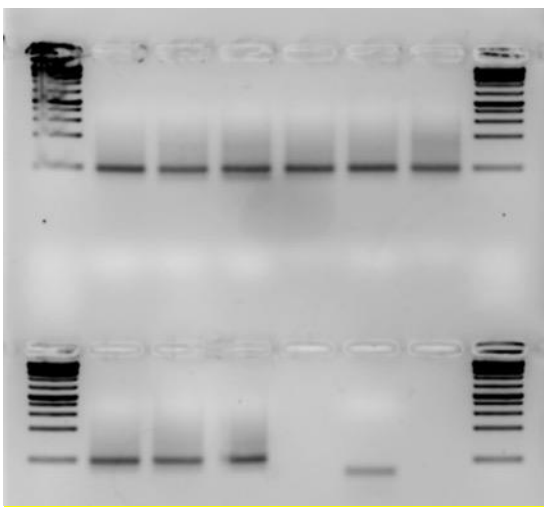


Figure 5-19 hsa-miR-652-3p FP1

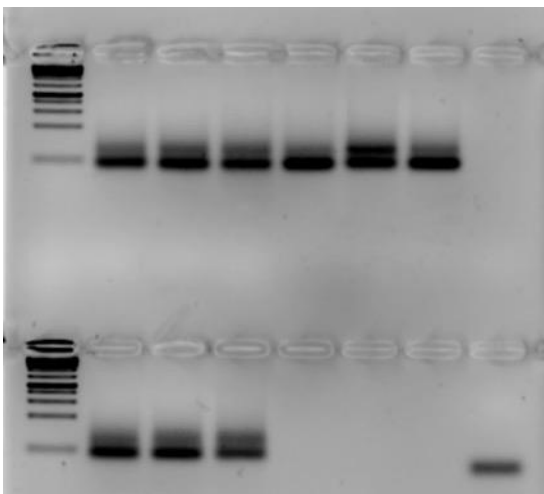


Figure 5-20 Histone H3 (Normalization control)

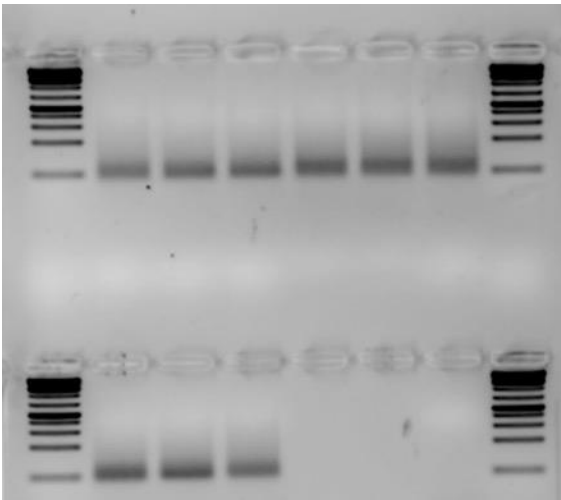


Figure 5-21 18s rRNA housekeeping gene

5.4.3 Validation of selected targets

After miRNA primers were selected and optimized, those demonstrating the highest selectivity and specificity for their target miRNAs were selected for further evaluation using RT-PCR, as described in Section 2.2.5.3 of the methodology. This validation process aimed to confirm the accuracy and reliability of the primers in detecting the intended miRNAs within the experimental cell lines. The results of this validation are presented below as individual miRNA FC expressions, normalised to OVSAHO (wildtype) which we deemed to be the control. This methods supports our hypothesis that there are miRNAs of prognostic value that are unique to *BRCA* status. Figure 4-22 and Figure 4-23 show the variation of the selected and validated miRNA expression profiles between OVSAHO (*BRCA* Wildtype) cell line compared with COV362 (*BRCA1* mutant) and Kuramochi (*BRCA2* mutant).

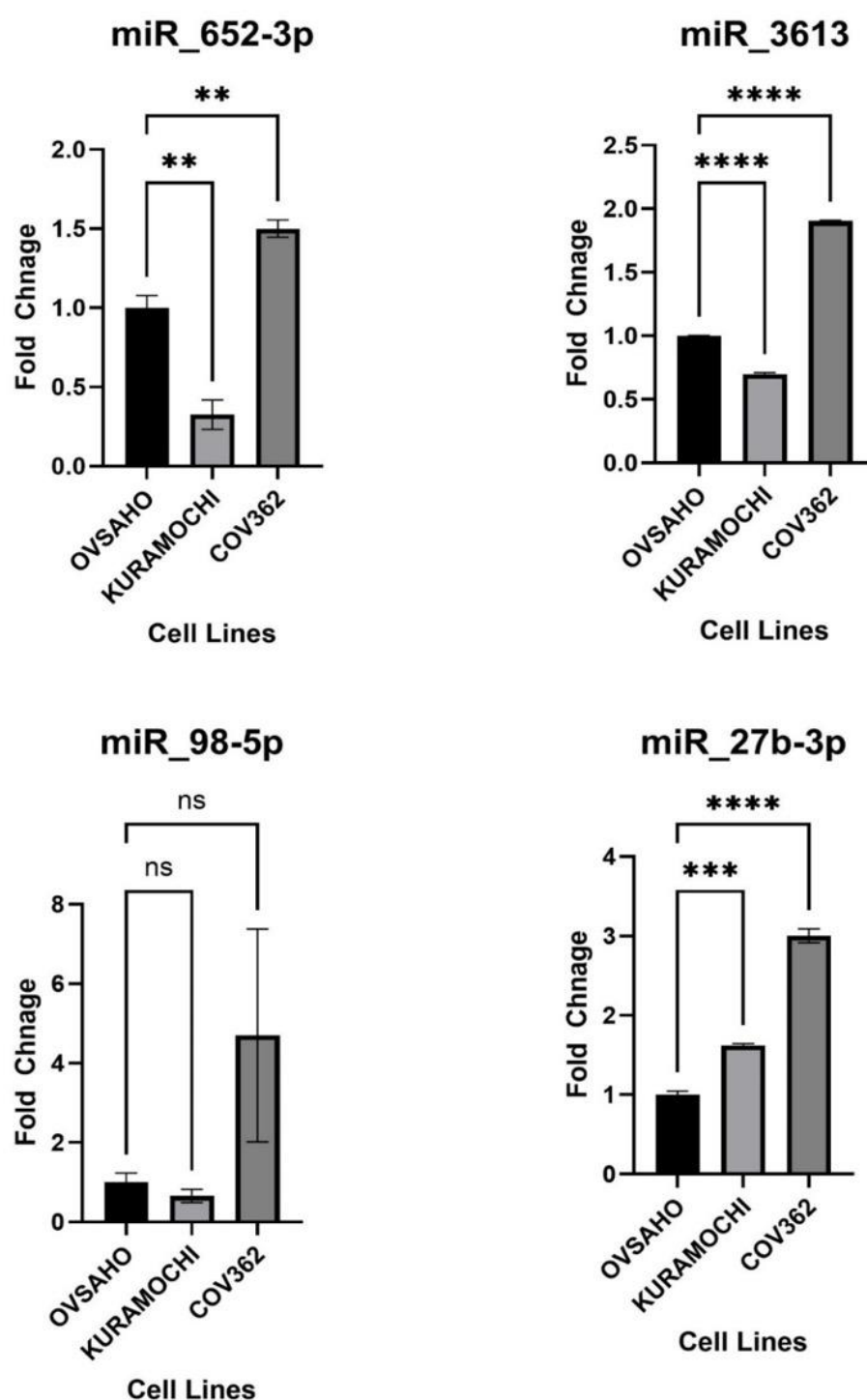


Figure 5-22 miRNA expression profiles between cell line

Relative expression of selected miRNAs was compared between *OVSAHO* (*BRCA*-wildtype), *KURAMOCHI* (*BRCA2*-mutant), and *COV362* (*BRCA1*-mutant) cell lines. Expression was normalised to the housekeeping control and presented as fold change relative to *OVSAHO*. Data are shown as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way ANOVA with multiple comparisons. Statistical significance is indicated as follows (GraphPad Prism, 2024): ns (not significant, $p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

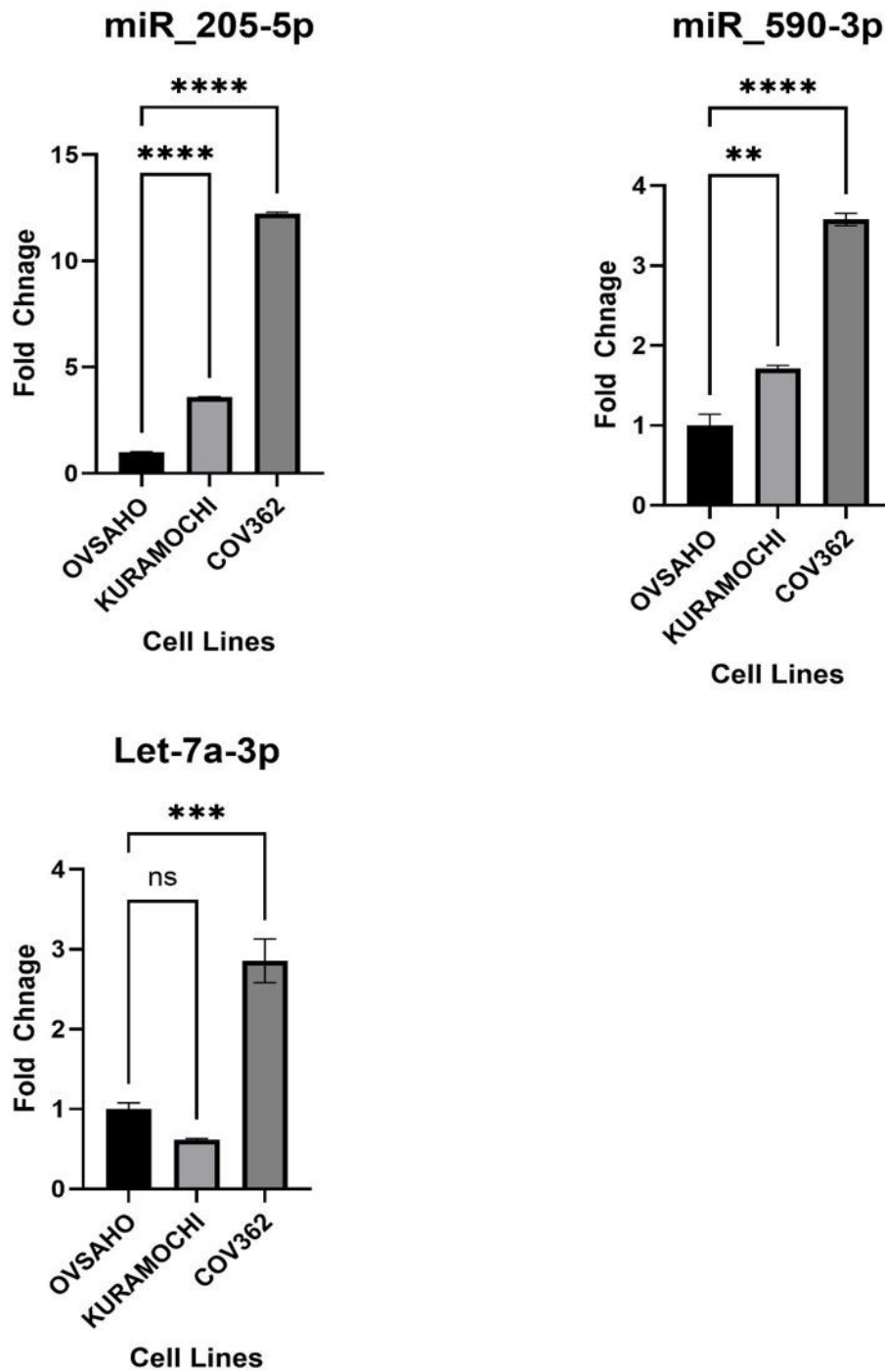


Figure 5-23 miRNA expression profiles between cell line

Relative expression of selected miRNAs was compared between *OVSAHO* (*BRCA*-wildtype), *KURAMOCHI* (*BRCA2*-mutant), and *COV362* (*BRCA1*-mutant) cell lines. Expression was normalised to the housekeeping control and presented as fold change relative to *OVSAHO*. Data are shown as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way ANOVA with multiple comparisons. Statistical significance is indicated as follows (GraphPad Prism, 2024): ns (not significant, $p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

The results showed that almost all miRNAs exhibited significant differential expression² between *BRCA* mutant and wild-type cells, as shown in Table 4-9. These results did not correlate with the predicted miRNA *BRCA* Status; however, interestingly, miRNAs were identified that were statistically significant for *BRCA* status. miR-205-5p demonstrated the highest statistical significance, with a p-value of less than 0.0001 (****) in both *BRCA1* and *BRCA2* mutant cell lines compared to wild type. miR-652-3p, miR-3613, miR-27b-3p, miR-205-5p, and miR-590-3p showed varying levels of significance across the KURAMOCHI and COV362 cell lines compared to OVSAHO. miR-98-5p remained non-significant ($P > 0.05$) across both cell lines, underscoring its lack of differential expression in *BRCA* mutant vs. wild-type cells.

Hypothesised miRNA by <i>BRCA</i> Status	miRNA	KURAMOCHI (<i>BRCA1</i> PV)	COV362 (<i>BRCA2</i> PV)
Common to all	miR_652-3p	**	**
Common to all	miR_3613	****	****
Unique to KURAMOCHI	miR_98-5p	Nos	Nos
Unique to KURAMOCHI	miR_27b-3p	***	****
Unique to KURAMOCHI	miR_205-5p	****	****
Unique to OVSAHO	miR_590-3p	**	****
Unique to OVSAHO	Let-7a-3p	Nos	***

Table 5-9 Significance of miRNA expression of *BRCA* mutant cell lines compared to the wildtype

Summary of hypothesised miRNAs across *BRCA1*-mutant (KURAMOCHI) and *BRCA2*-mutant (COV362) cell lines compared with *BRCA*-wildtype models. Statistical significance is indicated as follows (GraphPad Prism, 2024): Nos (not significant, $p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

5.5 Discussion

This study posited that specific prognostic miRNA expression profiles are associated with OC, with variations based on *BRCA* status. To investigate this hypothesis, extensive in silico analyses were conducted using various databases and online tools. These resources facilitated the identification of candidate miRNAs for validation in cell lines. The study specifically compared miRNA profiles across cell lines with differing *BRCA* mutation statuses. The aim of this was to highlight the role of prognostically relevant miRNAs as potential biological markers for *BRCA* mutation carriers.

5.6 Interpretation of Results

5.6.1 miR-652-3p

In this study, miR-652-3p showed significant upregulation in *BRCA*-mutant cells compared to wildtype, suggesting its potential as a marker for *BRCA*. miR-652-3p has been implicated in many cancers such as hepatocellular (366-368), bladder (369), prostate (370), colon (371), breast (372) and lung (373) cancer, and Chang et al. found miR-652 to be up-regulated in *BRCA1* mutant cells in mice models (374).

5.6.2 miR-3613

While miR-3613 has been linked to OC in several studies, its expression patterns vary. One study found significantly elevated levels of miR-3613 ($p < 0.05$) in OC patients compared to controls with associated endometriosis (375). Conversely, another study comparing primary and recurrent epithelial OC reported significantly lower miR-3613 levels in recurrent cases vs. primary, suggesting a possible association with disease recurrence. However, the genetic status (e.g., *BRCA* mutations) of these patient cohorts was unknown, leaving a gap in understanding how miR-3613 expression may vary by genetic background (376).

5.6.2.1 miR-98-5p

Despite miR-98-5p showing no significant difference in our study of *BRCA*-mutant vs. wild-type OC cells, evidence from other research points to its complex and sometimes conflicting roles in OC. Dong et al. reported that miR-98-5p acts as a tumour suppressor in OC, with overexpression leading to reduced cell proliferation, invasion, and EMT transition in SKOV-3 and A2780 cell lines (377). It's worth noting that both of these cell lines have been reported to be unlikely HGSOc, and neither are identified as having a *BRCA1* or *BRCA* (184). However, additionally, studies by Wang et al. (378), Guo et al. (379, 380) and Qi et al. found that miR-98-5p contributes to cisplatin resistance in OC, either by downregulating *Dicer1*, resulting in global miRNA suppression, or by targeting *CDKN1A*, leading to enhanced cell survival and chemoresistance. Its potential as a prognostic marker in OC remains uncertain; our findings suggest it does not seem to play a role in distinguishing *BRCA* status.

5.6.3 miR-27b-3p

Our study found that miR-27b-3p was significantly upregulated in both KURAMOCHI and COV362 OC cell lines compared to wild-type cells ($p \leq 0.001$ in KURAMOCHI and $p \leq 0.0001$ in COV362). miR-27b-

3p has well-established role in cancer progression across various types, including prostate, breast, gastric, colorectal, lung, glioma, cervical, hepatocellular carcinoma, thyroid, pancreatic, and oesophageal cancers (381-394). miR-27b-3p has been implicated in promoting tumour growth, migration, invasion, and metastasis by regulating key genes involved in cell cycle progression and apoptosis. Additionally, it has been linked to chemoresistance, with studies demonstrating its role in modulating resistance to chemotherapy and targeted therapies (395-401). These findings highlight the broad oncogenic role of miR-27b-3p and suggest its potential as a biomarker for aggressive tumour behaviour and treatment resistance, warranting further investigation into its specific impact on *BRCA*-mutant OC.

5.6.4 miR_205-5p

MiR-205-5p showed the most significant statistical difference, with a p-value of less than 0.0001 in both *BRCA1* and *BRCA2* mutant cell lines when compared to wild-type in our study. There are conflicting reports on the role of miRNA-205 in cancer in the literature. For example, there are studies which have reported an association between the overexpression of miR-205-5p with worse survival (402). Further studies suggest that miR-205 may have a tumour-suppression function (403). This tumour-suppression function may be explained by study which reported the miR-205 family have been shown to regulate E-cadherin transcriptional repressors, ZEB1 and SIP1 (ZEB2) (352), which in turn found that these miRNA-205 was markedly downregulated during EMT in invasive breast cancer cell lines. Additionally, Wu et al. (403) demonstrated that miR-205 inhibits cell growth and invasion, with Protein Kinase C-epsilon identified as a target, further supporting a tumour-suppressive role for miR-205, which is potentially enhanced by *BRCA1* upregulation. Several studies highlight the potential role of miR-205-5p in *BRCA* mutation carriers specifically. Gasparri et al. (404) reported that miR-205-5p is downregulated in breast cancer patients with wild-type *BRCA1* CAV compared to patients with a *BRCA1* CAV in excreted urine. This aligns with our findings demonstrating upregulation in *BRCA1/BRCA2*-mutant cells compared to wild-type. Tanic et al. (405) identified miR-205-5p as part of expression signatures predictive of *BRCA1/2* mutation-associated hereditary breast cancer and showed that miR-205 are induced in *BRCA1*-deficient HCC1937 cells after reconstitution with *BRCA1*, suggesting a possible overlap in their roles across cancer types. Chang et al (374) demonstrated miR-205-5p as targeted by *BRCA1* with upstream signal as shown in **Figure 5-24**. This is relevant as the set of miRNAs regulated by *BRCA1* indicates that the loss of *BRCA1* could result in the activation of oncogenic miRNAs or the suppression of tumour-suppressive miRNAs, which may contribute to cancer development (374).

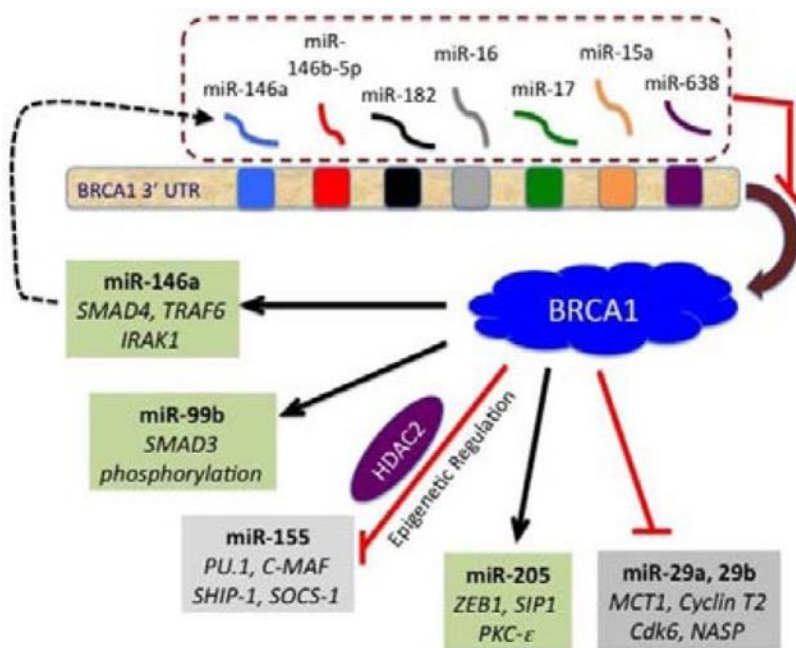


Figure 5-24 BRCA1-related miRNA network. Taken from Chang et al. (374).

5.6.5 miR_590-3p

The findings in our study, showing that miR-590-3p was significantly upregulated in both *BRCA1* and *BRCA2* mutant OC cell lines compared to wildtype. The role of miR-590-3p in cancer is complex as it has been described as both a tumour-suppressor gene in some cancers, and oncogenic in others (406). The oncogenic role of miR-590-3p in OC specifically has been previously described (407, 408). These studies demonstrate that miR-590-3p has been shown to target critical genes, such as FOXA2, which in turn regulates versican (VCAN), a gene associated with enhanced tumour growth and metastasis (407). Additionally, miR-590-3p has been found to regulate other oncogenic pathways by targeting cyclin G2 (CCNG2) and FOXO3, with effects on β -catenin signaling contributing to its tumour-promoting activity (408). While there has been no direct description of an association between miR-590-3p and *BRCA* mutations, the role of miR-590-3p in modulating key oncogenic pathways suggests that its dysregulation in *BRCA1/2* mutant OC could contribute to an enhanced aggressive phenotype. Further studies are needed to explore its potential as a therapeutic target in *BRCA*-mutated OC.

5.6.5.1 Let-7a-3p

Our findings showed that Let-7a-3p was significantly upregulated in *BRCA2* mutant OC cell lines compared to wild-type, with a p-value of <0.001. The let-7 miRNA family is commonly downregulated in various cancers, and its altered expression could serve as a potential biomarker for predicting patient response to therapy. García-Vázquez et al. (137) found that let-7a-3p was significantly upregulated in OC tumours compared to normal ovarian tissues, although no direct link to chemotherapy response was established in their study. However, the observed upregulation of let-7a-3p in *BRCA2* mutant cells suggests it may play a role in influencing treatment sensitivity, and

further research is needed to explore its potential contribution to OC progression and response to therapy.

5.6.6 miRNA and *BRCA*

There is a number of articles, both original and review, that have explored and summarised the role of miRNAs in the context of *BRCA* mutations, particularly their involvement in cancer progression and potential as biomarkers for therapeutic response (374, 402). In line with the inherent complexity of miRNA research, there appears to exist a bidirectional relationship between miRNA and *BRCA*, where certain miRNAs are regulated by *BRCA*, and conversely, *BRCA* expression can also be modulated by miRNAs.

BRCA1/2 gene expression can be modulated by various miRNAs, creating a "BRCAness-like" effect by downregulating key DNA damage response elements (402). For example, miR-302b indirectly impairs *BRCA1* function through E2F1 and ATM downregulation (409), while other miRNAs, such as miR-146a (410), miR-9 (411), and miR-182 (412), bind to *BRCA1/2* mRNAs, reducing expression and increasing sensitivity to DNA damage. Other miRNA, such as miR-155 (413, 414) and miR-205,(352) have been shown to be regulated by *BRCA1* (404). However, it is worth noting, that many of the studies on this topic primarily focus on breast cancer (402, 405, 415, 416) rather than OC. A study by Elias et al. analysed serum miRNA profiles from 653 healthy participants across six international cohorts and identified a set of 10 miRNAs—hsa-miR-20b-5p, hsa-miR-19b-3p, hsa-let-7b-5p, hsa-miR-320b, hsa-miR-139-3p, hsa-miR-30d-5p, hsa-miR-17-5p, hsa-miR-182-5p, hsa-miR-421, and hsa-miR-375-3p—that could distinguish between *BRCA*-mutation carriers and wild-type individuals (417). The primary aim of this study was to identify healthy individuals at high risk for *BRCA1/2* mutations before the development of cancer. In contrast, the potential role of identifying miRNA in OC patients would be to use it as a prognostic biomarker, as well as a potential indicator for eligibility for treatment.

As outlined above in section 5.6.4, Tommasi et al. (402) demonstrated that miR-205-5p is downregulated by wild-type *BRCA1*, suggesting that miR-205-5p may act as a modulator of the *BRCA1* pathway. Further, Tanic et al. (405) identified specific miRNA expression signatures in *BRCA1/2* mutation-associated breast cancers, showing that these miRNAs could predict the clinical behaviour of hereditary breast cancers. These miRNAs included miR-142-3p, miR-505, miR-1248, miR-181a-2, miR-25, and miR-340, which were able to differentiate between *BRCA1/2* mutation carriers and noncarriers with high accuracy.

5.7 Limitations

The methodology employed in this study presents several limitations outlined below.

5.7.1 Limitations of In Silico Work

In silico approaches, such as those using tools like TargetScan (258) and KM Plotter (257), have been instrumental in identifying potential miRNA targets and their association with clinical outcomes. However, these computational methods rely heavily on available databases and prediction algorithms, which have several limitations that could impact the reliability and relevance of the findings (418).

One major limitation is the inherent complexity of miRNA regulation, which may not be fully captured by in silico tools. miRNAs typically regulate gene expression through binding to the 3' untranslated region (UTR) of target mRNAs, but the interaction is often influenced by a variety of factors such as RNA secondary structure, the presence of RNA-binding proteins, and tissue-specific contexts (419). Tools like TargetScan are based on conserved miRNA target sites across species, which may not always accurately reflect the dynamics of miRNA-mRNA interactions in humans, particularly in the context of cancer (420). Additionally, many in silico tools fail to account for post-transcriptional modifications and the interplay between miRNAs and other non-coding RNAs or regulatory molecules (421), which may significantly influence miRNA function.

Moreover, in silico predictions are often limited by the datasets used for training the algorithms. The sample sizes in many publicly available datasets, such as those used in TargetScan and KM Plotter, may be relatively small or unrepresentative of the diverse patient populations (421). As a result, the identified miRNA-mRNA interactions or survival associations may not be universally applicable, limiting the generalizability of the findings (420). Inconsistent or low-quality data across different datasets can further complicate the interpretation of results. The accuracy of miRNA-target predictions is also contingent on the quality of annotations in these databases, which may not always be up-to-date or comprehensive, leading to potential false positives or negatives (422).

5.7.2 Limitations of Cell Line Experiments

Cell line experiments are commonly used to investigate the biological relevance of miRNAs in cancer, including their roles in regulating gene expression, tumorigenesis, and response to treatment. However, there are several limitations when translating in vitro findings from cell line models to human disease.

First, cell lines may not fully recapitulate the heterogeneity and complexity of primary tumour tissues. Cancer cell lines, especially those immortalized for extended use in laboratories, can undergo significant genetic and phenotypic changes that may not reflect the original tumour from which they were derived (423). As a result, the expression profiles of miRNAs and their targets in cell lines might differ significantly from those in patient tissues, reducing the applicability of the results. Moreover,

cell lines often lack the stromal components, immune cells, and other microenvironmental factors that play a crucial role in cancer progression (424), which can influence miRNA expression and function.

Another limitation of cell line work is the potential variability in experimental conditions, such as differences in cell culture media, passage number, and handling procedures. These factors can introduce significant variability into the results, making it difficult to reproduce findings across different laboratories or even within the same laboratory (425). Variability in assay sensitivity, particularly when measuring miRNA expression levels, can also impact the reliability of results, leading to inconsistent findings regarding miRNA-target interactions or their roles in cancer progression (426).

5.7.3 Challenges in Identifying Clinically Relevant Targets

One of the overarching challenges in miRNA research, whether through in silico work as outlined above, or cell line work, is the complexity of miRNA networks. miRNAs do not function in isolation but are part of intricate regulatory networks that interact with a multitude of target genes (426). These networks are context-dependent, meaning that the same miRNA may have different effects in different tissues, stages of disease, or response to therapies. As a result, identifying miRNAs that are consistently associated with clinical outcomes, such as *BRCA* status or cancer prognosis, is difficult.

In conclusion, while in silico tools and cell line models provide valuable insights into the potential roles of miRNAs in cancer, the complexity of miRNA regulation and the limitations of these experimental approaches highlight the need for further validation in patient samples and more refined models. The integration of additional layers of data, such as genomic, transcriptomic, and proteomic information, will be crucial in overcoming these challenges and improving the clinical applicability of miRNA-based biomarkers.

5.7.4 Potential Clinical Application

The results of this study highlight several miRNAs, particularly miR-205-5p, which demonstrated significant differential expression between *BRCA* mutant and wild-type OC cell lines. These findings suggest the potential for miRNAs to serve as biomarkers for *BRCA* mutation status, which could have clinical implications in personalized treatment approaches. However, it is important to note that extensive validation in larger, more diverse patient cohorts is needed before these miRNAs can be considered for clinical application. Further research is necessary to confirm their utility and ensure reliable, reproducible results across different stages and patient populations. Ultimately, this research serves to contribute to the growing body of knowledge in the field, advancing our understanding of miRNA biomarkers in OC and their potential clinical applications.

Exploring the Relationship between *BRCA* status and circRNA in OC

Chapter Six

6 Exploring the Relationship between BRCA and circRNA

6.1 Introduction

Over the past two decades, the advent of high-throughput sequencing technologies has significantly advanced the understanding of genetic mutations and their implications in various cancers (427).

Since their identification in the mid-1990s, mutations in the *BRCA1* and *BRCA2* genes have been well-known for their roles in increasing the risk of breast, ovarian and other cancers (428).

Simultaneously, the discovery of circRNAs, in the early 2010s has added a new dimension to the complex regulatory networks within cells (429). CircRNAs have been implicated in numerous biological processes and diseases, including cancer, due to their ability to regulate gene expression and protein function. Further, recent evidences have demonstrated that circular RNAs play a crucial role in fine tuning the level of miRNA mediated regulation of gene expression by sequestering the miRNAs (222). Their interaction with disease associated miRNAs indicates that circular RNAs are important for disease regulation (190).

This chapter explores the previously uncharacterised relationship between *BRCA* status and circRNA expression using both experimental and bioinformatic approaches. The investigation begins with the careful selection of a comprehensive cell line model, including OC lines with a *BRCA1* mutation, *BRCA2* mutation and *BRCA* wildtype to ensure a thorough and comparative analysis. The cell lines were then characterized and verified to confirm their genetic profiles. Subsequently, RNA was processed to identify both upregulated and downregulated circRNAs, and extensive bioinformatics analyses were conducted to identify circRNAs of interest. Selected circRNAs were further investigated by designing custom primers and validating their expression in the cell lines. Additionally, the circRNAs of interest were evaluated in a panel of cell lines and in tissue samples from OC patients with varying *BRCA* statuses.

Through this exploration, the chapter seeks to identify circRNAs that could serve as biomarkers for *BRCA* mutations. By assessing the differential expression of circRNAs in cell lines with distinct *BRCA* genetic profiles, the study aims to pinpoint circRNAs that are specifically associated with *BRCA* mutations. These circRNAs could potentially be developed into biomarkers for *BRCA*-related cancers and therapeutic targets, thereby contributing to more effective cancer diagnostics and treatments.

6.2 Aims and Objectives

6.2.1 General Aims

This chapter aims to assess circRNA expression in relation to *BRCA* status, specifically in the context of OC. The potential pathways by which circRNAs may influence the expression and function of *BRCA* genes, impacting OC development and progression are explored.

6.2.2 Hypothesis

The hypothesis is that circRNAs are involved in regulating *BRCA* genes, with variations in circRNA profiles dependent on *BRCA* status.

6.2.3 Objectives

The objective of this chapter was to;

- Identify and select cell line models that closely replicate the clinical environment
- Assess circRNA expression in relation to *BRCA* status, specifically in the context of OC
- Validate the regulatory roles of circRNAs through translational studies using samples from patient cohorts.

6.3 Results

6.4 Cell Line Selection, Verification and Characterisation

6.4.1 Cell Line Selection

To address the hypothesis that circRNA differ between various *BRCA* statuses, specific cell lines were carefully selected to represent HGSOC and reflect different *BRCA* status. The cell lines chosen are outlined below (**Table 6-1**). Ensuring the quality and authenticity of these cell lines was paramount; a literature review was undertaken to confirm their validity as HGSOC models. This included consulting studies that ranked OC cell lines by their suitability for HGSOC modelling, in which the selected cell lines ranked 1st, 2nd, and 4th overall, and all fell within the top 20 most likely to represent true HGSOC based on transcriptional profiling (46, 184)

	Cell Line	COV362	KURAMOCHI	OVSAHO
Quality Assurance	Ranking position of 47 OC cell lines by suitability as HGSOC models (184)	Ranked no 4 out of 47 OC cell lines	Ranked no 1 out of 47 OC cell lines	Ranked no 2 out of 47 OC cell lines
	Likelihood to represent HGSOC (46)	Within Top 20 most likely HGSOC	Within Top 20 most likely HGSOC	Within Top 20 most likely HGSOC
Cell line Characteristics	Derived from	Metastatic; Pleural effusion	Metastatic; Ascites	Metastatic; Abdomen
	<i>TP53</i> Status	Mutation	Mutation	Mutation
	<i>BRCA</i> status	<i>BRCA1</i> Mutation	<i>BRCA2</i> Mutation	No <i>BRCA1</i> / <i>BRCA2</i> mutation. <i>BRCA2</i> homozygous deletion

Table 6-1 Cell Line Selection

6.4.2 Cell Line Morphology

6.4.2.1 COV362

COV362 is a *BRCA1* mutant HGSOC cell line derived from malignant pleural effusion. It typically exhibits a polygonal and fusiform morphology, which is characteristic of epithelial cell lines (**Figure 6-1, Figure 6-2**).

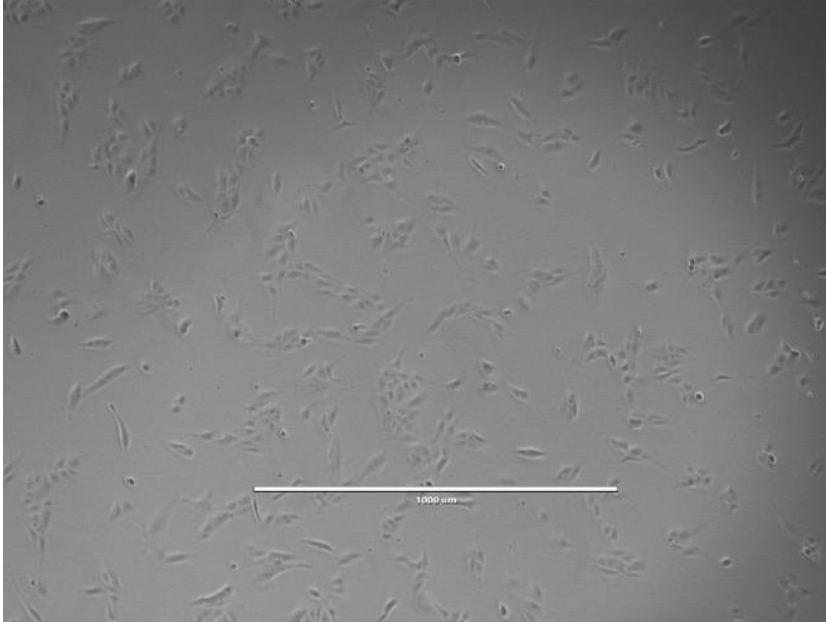


Figure 6-1 Cov362 photographed at 4x magnification

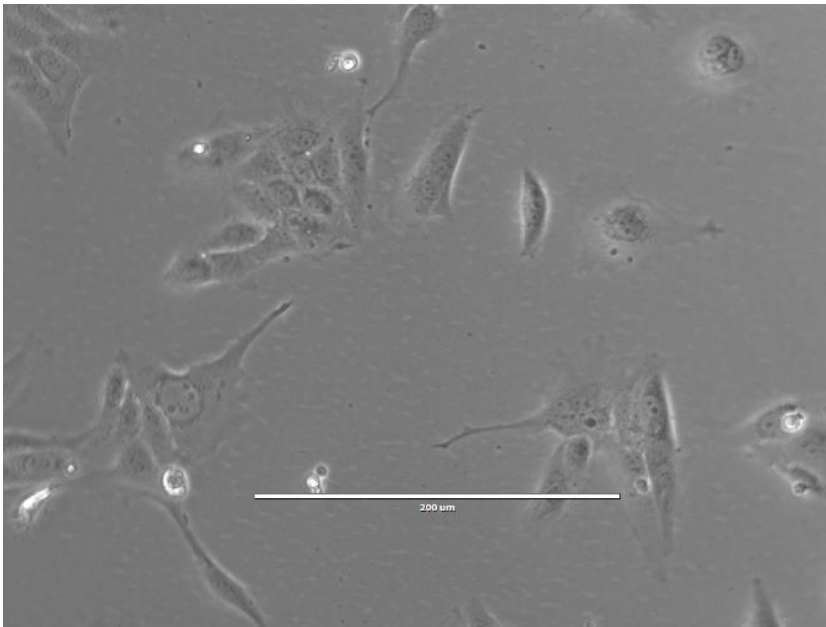


Figure 6-2 Cov362 photographed at 40x magnification

6.4.2.2 OVSAHO

OVSAHO is a *BRCA* wild-type HGSOC cell line derived from metastatic abdominal tissue. It typically exhibits an epithelial-like cobblestone morphology (**Figure 6-3,**

Figure 6-4).

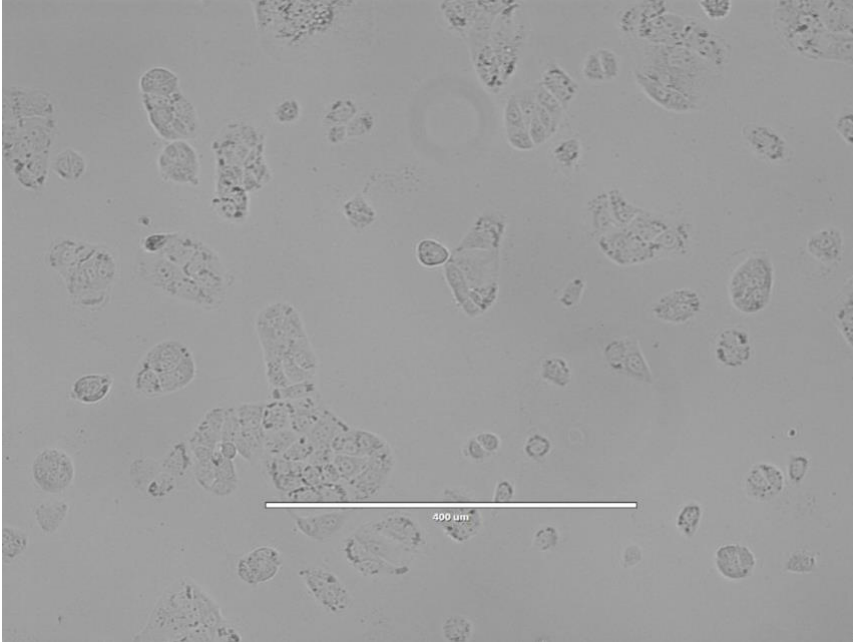


Figure 6-3 OVSAHO photographed at 10x magnification

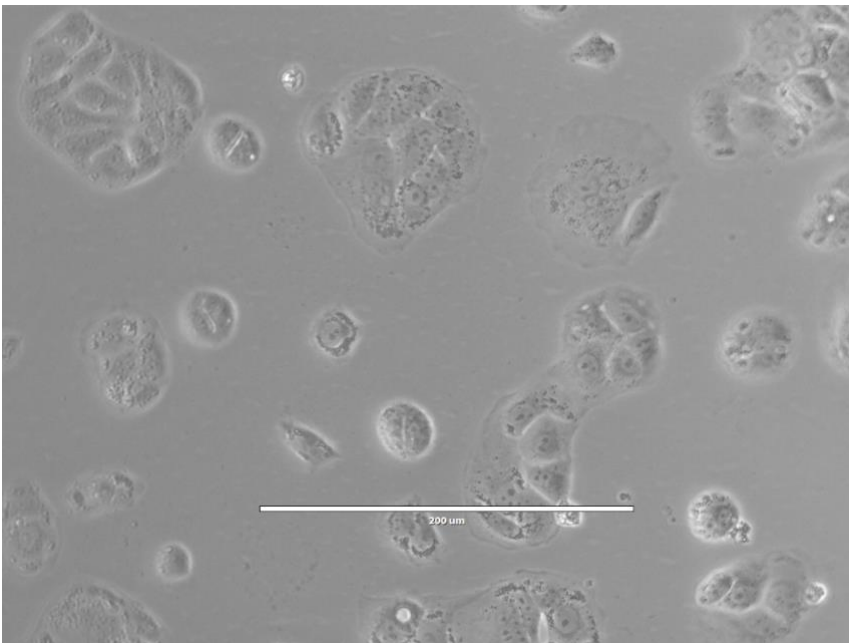


Figure 6-4 OVSAHO photographed at 40x magnification

6.4.2.3 KURAMOCHI

Kuramochi is a *BRCA2* mutant HGSOC cell line derived from malignant ascites. It typically exhibits a fibroblastic morphology (**Figure 6-5, Figure 6-6**).

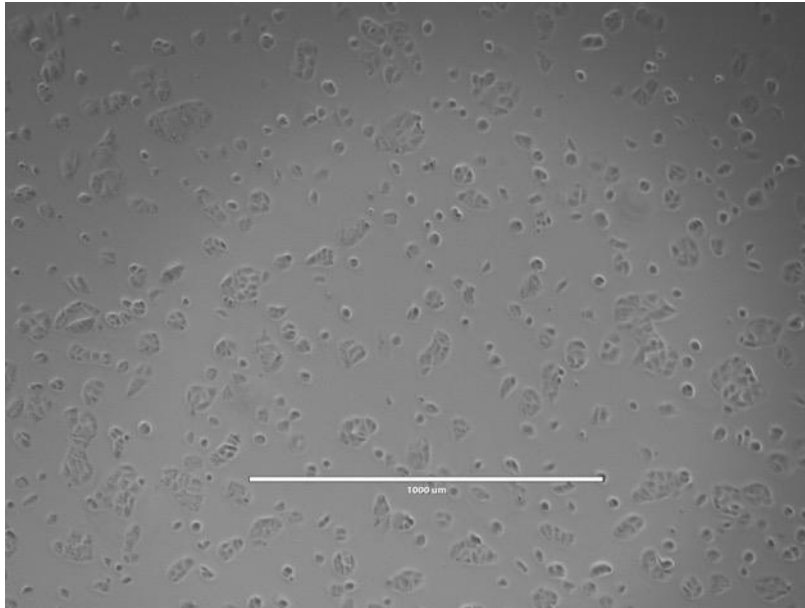


Figure 6-5 Kuramochi photographed at 4x magnification

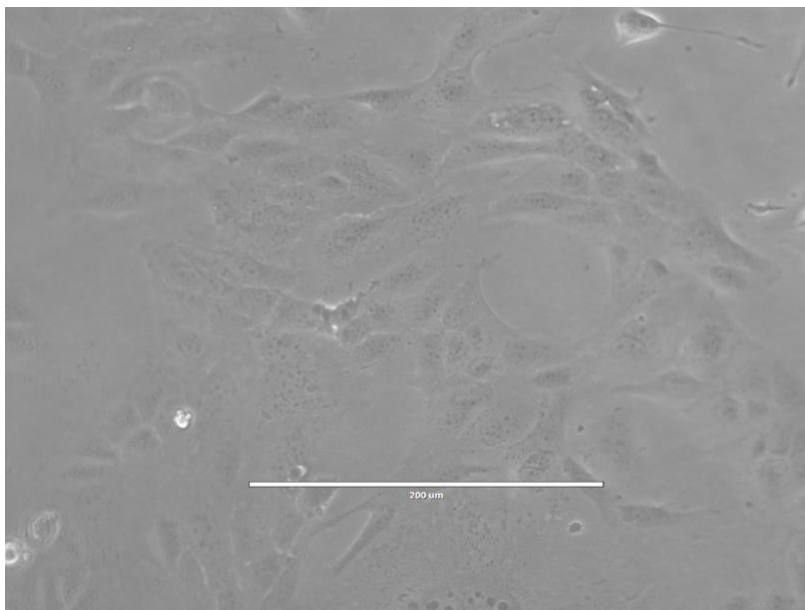


Figure 6-6 Kuramochi photographed at 40x magnification

6.4.3 Mycoplasma testing

Cells were tested for mycoplasma infection within four weeks and every 16 weeks thereafter. A PCR-based assay was performed using specific primers for common mycoplasma species, with reactions conducted over 40 cycles using the 7500 Fast Real-Time PCR System as per [Section 2.4.6](#). COV362 demonstrated a positive as indicated by a band at 270 bp (**Figure 6-7**).

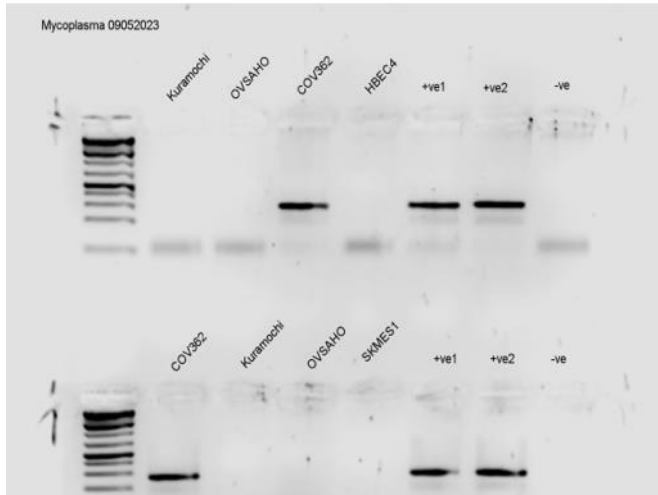


Figure 6-7 Mycoplasma Gel Showing COV362 Positivity

6.4.4 Mycoplasma Clearance

COV362 was treated with 60 μ L of Plasmocure (100 mg/mL) for 17 days, during which time mycoplasma testing was performed twice weekly until the infection was no longer detected (**Figure 6-8**). Thereafter, the cell line was retested periodically for mycoplasma throughout the remainder of the study.

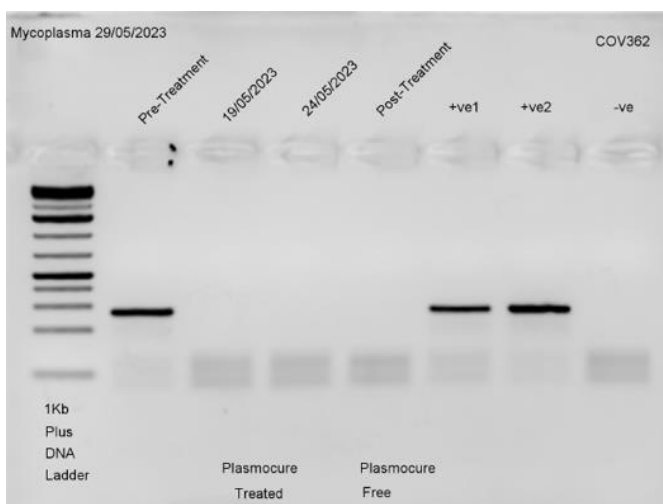


Figure 6-8 Pre and Post treatment mycoplasma PCR results for COV362 cell line

6.4.5 Cell Line Authentication

Cell lines were authenticated using the Human Cell Line Authentication service from Eurofins Genomics. KURAMOCHI and OVSAHO were confirmed as exact matches to their respective reference profiles. COV362 was also identified as the stated cell line, although minor discrepancies were noted. Given the high overall similarity (>95%), the cell line was considered suitable for inclusion in downstream analyses, with appropriate caution exercised in interpretation (**Figure 6-9**).

Client Sample Name	KURAMOCHI	COV362	OVSAHO
Sample Code	CL00014407	CL00014408	CL00014409
D8S1179	10,11	11,11	14,14
D21S11	30,32.2	31,32.2	31,31
D7S820	10,11	10,14	8,10
CSF1PO	11,12	11,12	10,12
D3S1358	18,18	16,18	15,15
TH01	9,9	9.3,9.3	6,6
D13S317	9,12	14,14	8,8
D16S539	10,10	11,11	9,9
D2S1338	18,18	24,25	22,23
D19S433	13,14	14,14	15.2,16.2
vWA	16,19	15,18	14,16
TPOX	8,12	11,11	8,11
D18S51	13,13	13,13	16,20
AMEL	X,X	X,X	X,X
D5S818	12,12	13,14	12,13
FGA	21,23	23,23	24,24
Database Name	KURAMOCHI	COV362*	OVSAHO

Figure 6-9 Human Cell Line Authentication Summary

6.4.6 BRCA Characterisation and Verification

As outlined in Section 2.1.3.2, a comprehensive *BRCA* status verification was performed on three distinct cell lines: OVSAHO, COV362, and KURAMOCHI. The *BRCA1* and *BRCA2* genes were analysed for PVs. The purpose of this verification was to identify the presence or absence of mutations that could influence the cellular behaviour related to cancer development and treatment response. The verification process involved detailed genetic analysis, including sequencing techniques to detect specific mutations. The results of this analysis are presented in **Table 6-2**. Each cell line's *BRCA1* and *BRCA2* status is documented, highlighting any detected mutations, their locations (exons), variant allele frequencies (VAF), and the predicted impacts on protein function.

Cell Line	<i>BRCA1</i>	<i>BRCA2</i>
OVSAHO	No mutation detected	No mutation detected
COV362	<i>BRCA1</i> E10 90% VAF c.2612delinsTT p.Pro871Leufs*32	No mutation detected
	<i>BRCA1</i> E10 100% VAF c.4096+1G>T	
KURAMOCHI	No mutation detected	<i>BRCA2</i> E13 72% VAF c.6952C>T p.Arg2318Ter*

Table 6-2 *BRCA1* and *BRCA2* Mutation Status in OC Cell Lines

*Possibly a second low level variant at 5% for this sample but might be artefact *BRCA2* E11 4.9% VAF
c.1992delG p.Thr665Glnfs*3

6.4.7 RNA Quality Check

Following verification and classification of the cell lines, RNA was extracted and assessed for quality to enable downstream circRNA analysis. Electrophoresis of RNA samples (**Figure 6-10**) demonstrated distinct bands corresponding to the 18S and 28S ribosomal subunits, indicating good RNA integrity. The clear presence of these bands confirmed that the RNA samples were of high quality and suitable for subsequent analysis.

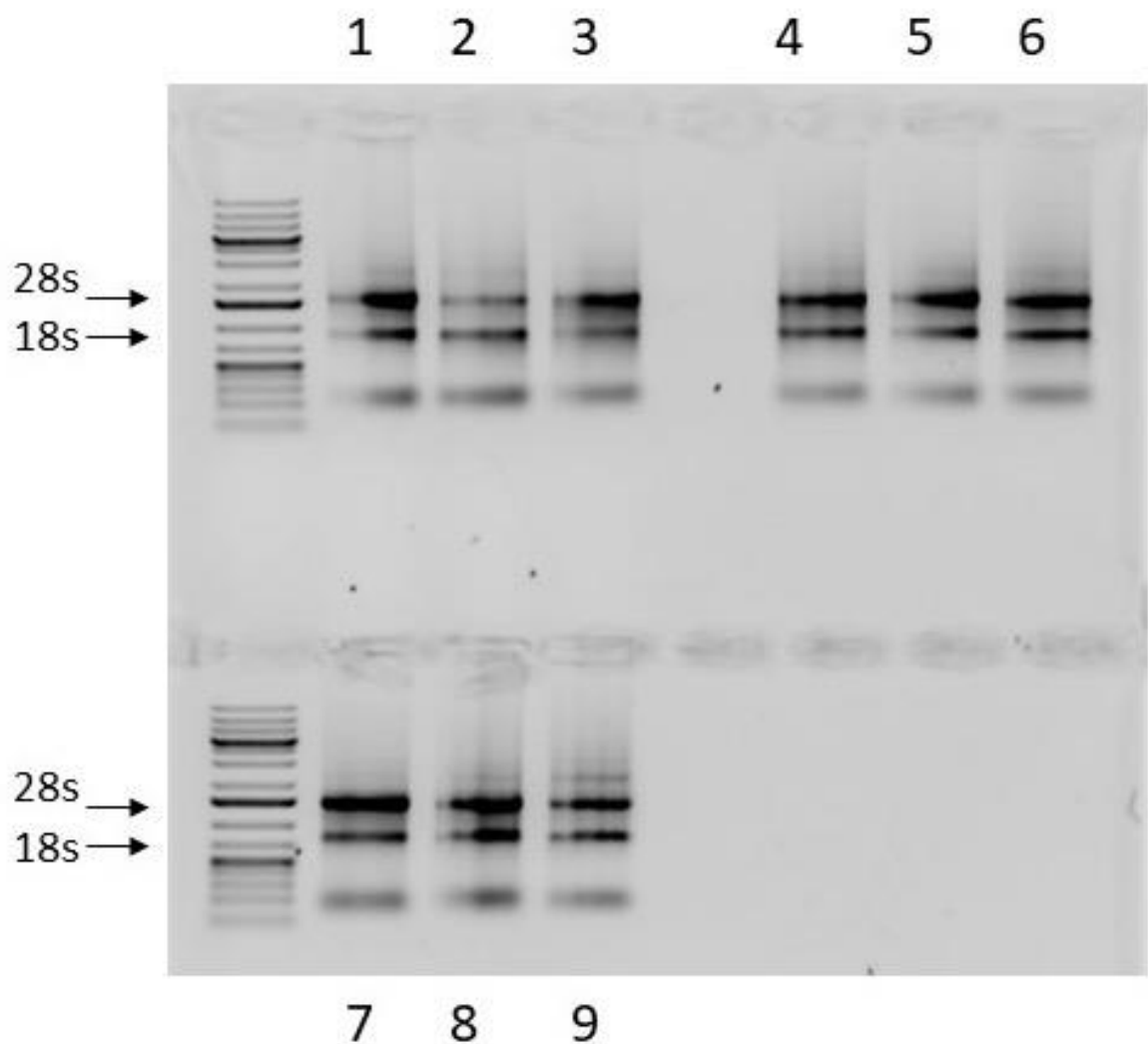


Figure 6-10 RNA Integrity Confirmed by 18S and 28S Bands

Lanes 1–3 correspond to OVSAHO, lanes 4–6 to KURAMOCHI, and lanes 7–9 to COV362 cell lines.

6.5 Visualisation of circRNA Expression Patterns

6.5.1 Hierarchical Clustering Heatmaps

Hierarchical clustering heatmaps were generated to explore circRNA expression patterns across selected HGSOC cell lines using unsupervised clustering analysis. This included COV362 (*BRCA1* mutant), OVSAHO (*BRCA* wildtype), and KURAMOCHI (*BRCA2* mutant) (**Figure 6-11**). Across the full dataset, clustering patterns appeared largely random, with no consistent grouping by *BRCA* status or cell line (red indicates high expression, green indicates low expression, and black represents neutral or baseline expression levels). This suggests that expression of the target circRNAs may not be primarily driven by *BRCA* genotype, or that confounding factors such as biological variability, post-transcriptional regulation, or technical noise may obscure underlying trends. While no global patterns were evident on heatmaps, it remains feasibility that individual circRNAs which act as a biomarker for *BRCA* exist, but are masked by background variability, highlighting the need for further validation.

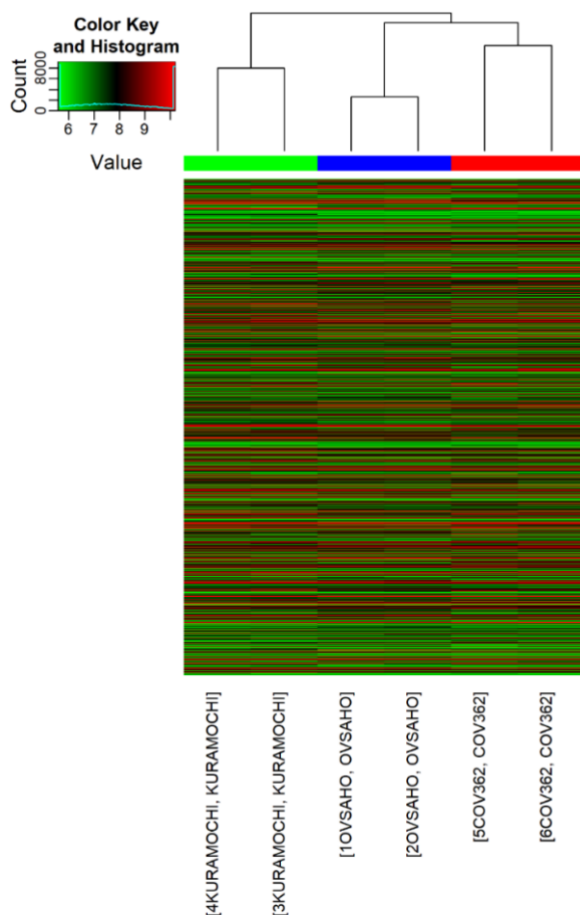


Figure 6-11 Hierarchical Clustering Heatmaps of all target values

To further investigate, pairwise heatmaps were generated for key comparisons: *BRCA1* mutant (COV362) vs. wildtype (OVSAHO), *BRCA2* mutant (KURAMOCHI) vs. wildtype, and *BRCA1* vs. *BRCA2*. In the comparison of COV362 vs. OVSAHO, samples clustered clearly by cell line, with reciprocal circRNA expression patterns across two main clusters, suggesting cell line-specific rather than *BRCA1*-driven effects (**Figure 6-12**). Similarly, the KURAMOCHI vs. OVSAHO heatmap showed clustering by cell line without a clear expression signature attributable to *BRCA2* status (**Figure 6-13**). In the direct comparison between COV362 and KURAMOCHI (*BRCA1* vs. *BRCA2*), clustering did not align with *BRCA* mutation class, and expression profiles remained heterogeneous (**Figure 6-14**). Although consistent global patterns were not observed across comparisons, the presence of variably expressed circRNAs indicates potential candidates for further study. These findings underscore the complexity of circRNA regulation in HGSOC and support the use of complementary validation strategies in larger or clinically annotated datasets.

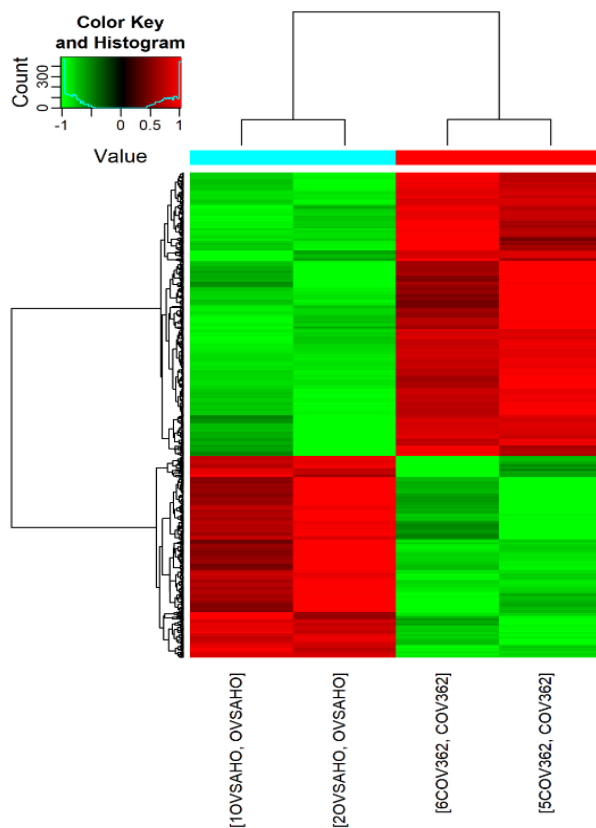


Figure 6-12 Hierarchical Clustering Heatmaps COV362 vs. OVSAHO

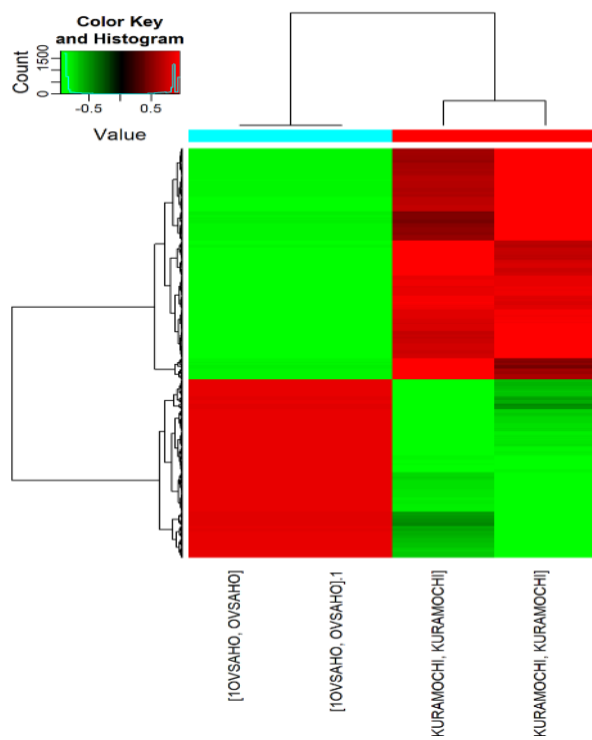


Figure 6-13 Hierarchical Clustering Heatmaps KURA vs. OVSAHO

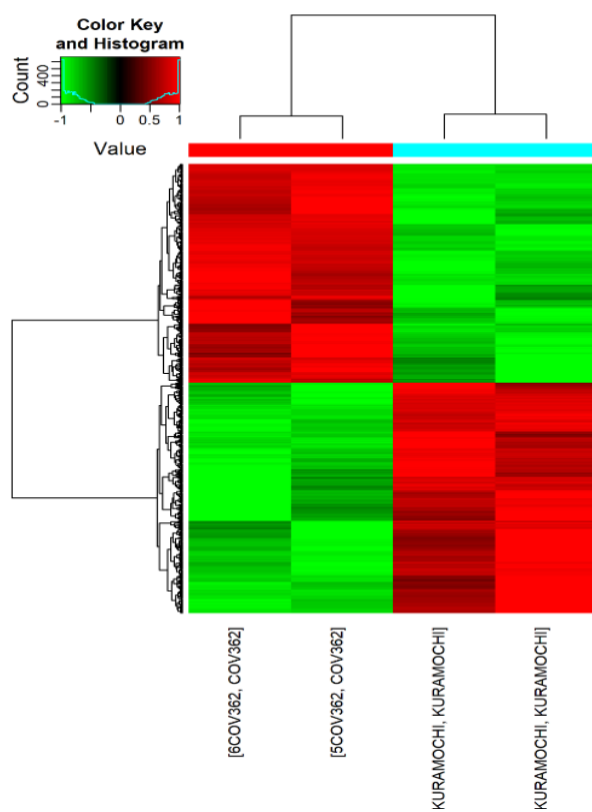


Figure 6-14 Hierarchical Clustering Heatmaps COV362 vs. KURA

6.5.2 Scatter Plots

The Scatter plots were generated to visualise differential circRNA expression across three key pairwise comparisons: COV362 (*BRCA1* mutant) vs. OVSAHO (*BRCA* wildtype) (**Figure 6-15, A**), COV362 vs. KURAMOCHI (*BRCA2* mutant) (**Figure 6-15, B**), and KURAMOCHI vs OVSAHO (**Figure 6-15, C**). Each axis represents the averaged, log2-normalised signal intensity for a given comparison, allowing visualisation of the relative abundance of circRNAs across cell lines. Across all comparisons, the majority of circRNAs clustered along the diagonal, suggesting broadly similar expression levels between cell lines, with a smaller subset of circRNAs showing marked up- or downregulation. This pattern supports the notion that while most circRNAs are similarly expressed across models, a distinct subset may exhibit cell line- or BRCA-specific expression changes. These findings are broadly consistent with the heatmap data (**Figure 6.12–6.14**).

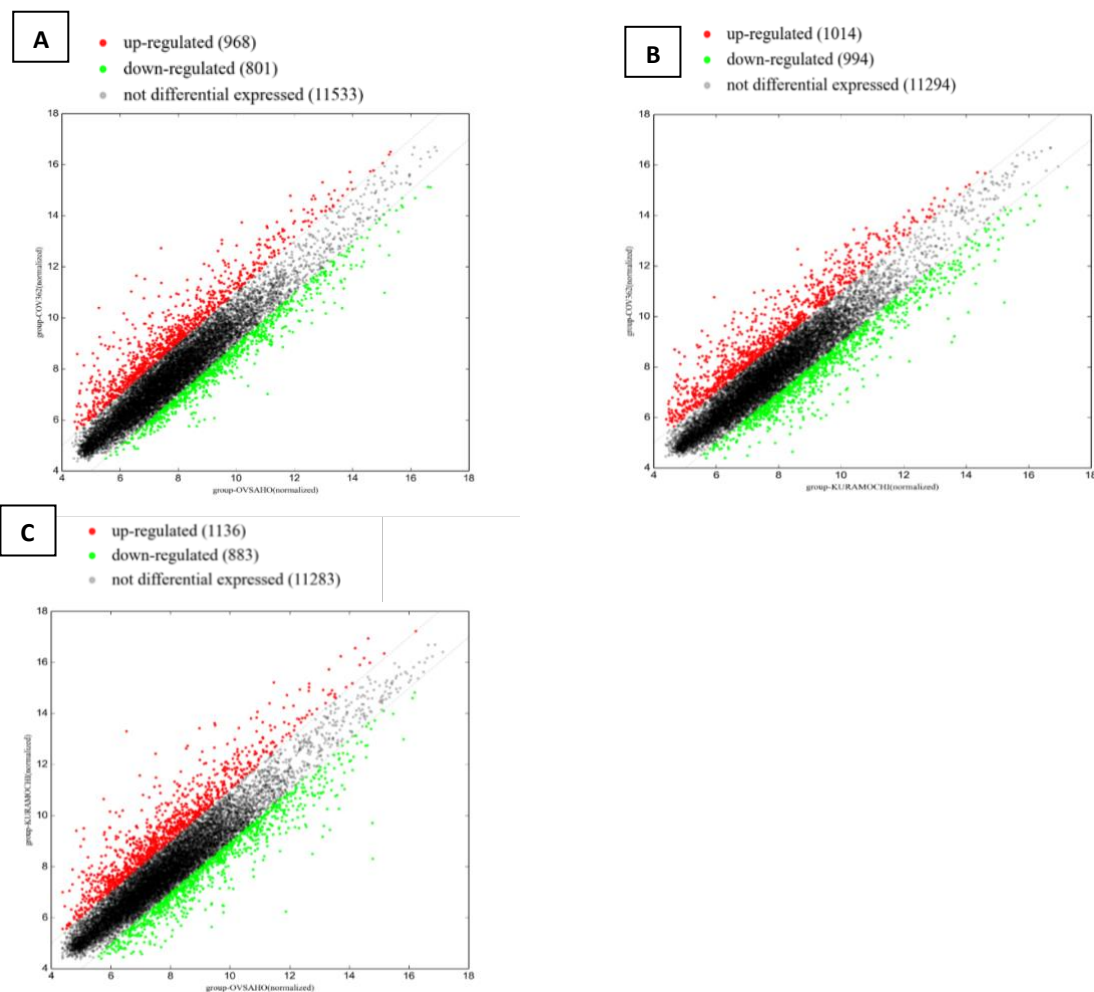


Figure 6-15 Scatter plots of circRNA expression in BRCA-related cell line comparisons

6.5.3 Volcano Plots

Similarly, the volcano plots provide a detailed view of differential circRNA expression across three crucial comparisons: KURAMOCHI vs. OVSAHO (**Figure 6-16, A**), COV362 vs. OVSAHO (**Figure 6-16, B**), and COV362 vs. KURAMOCHI (**Figure 6-16, C**). As expected from cell lines of shared histological origin (HGSOC), the majority of circRNAs showed similar expression patterns, reflected in the dense clustering of points around the centre of each plot. However, several circRNAs were clearly upregulated (red) or downregulated (green) between specific cell line pairs. These differentially expressed circRNAs represent candidates of interest for further investigation in the context of *BRCA*-related molecular pathways in OC.

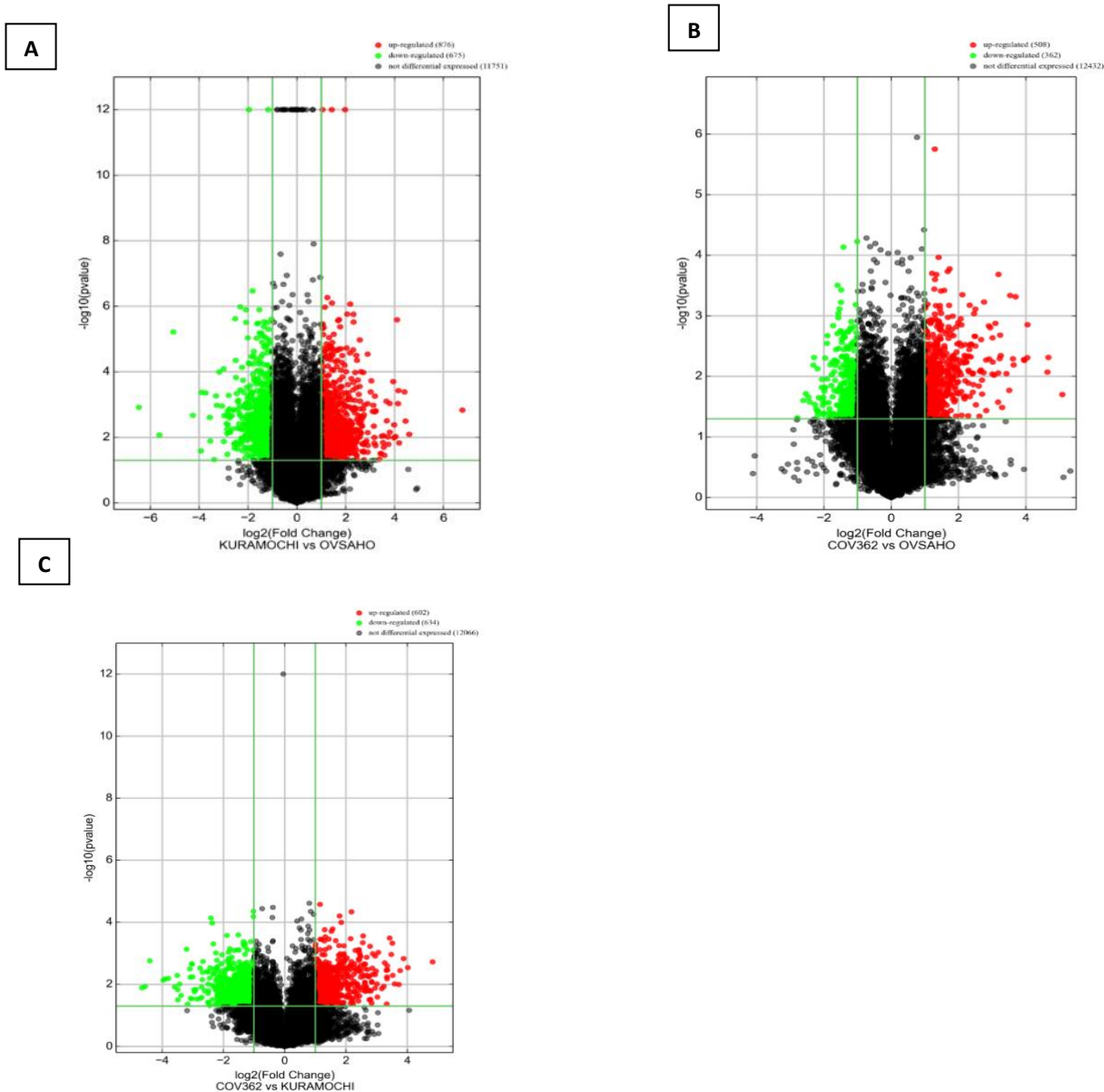


Figure 6-16 Volcano plots of circRNA expression in *BRCA*-related cell line comparisons

6.5.4 Gene Ontology Enrichment in *BRCA* Linked circRNAs

To explore the functional impact of differentially expressed circRNAs in *BRCA* related OC, Gene Ontology (GO) enrichment analysis was performed across key pairwise comparisons. The analysis

focused on circRNAs that were significantly upregulated, as these are more stable, more likely to have functional roles, and generally easier to detect and validate experimentally. GO enrichment plots were used to identify overrepresented biological processes (BPs), molecular functions (MFs), and cellular components (CCs) associated with these circRNAs.

6.6 GO Enrichment in COV362 vs. OVSAHO

6.6.1 Biological Processes (BPs)

GO enrichment analysis of circRNAs upregulated in COV362 (*BRCA1* mutant) compared to OVSAHO (*BRCA* wildtype) revealed significant enrichment of BPs related to signalling, development, and neurogenesis (**Figure 6-17**). The top enriched GO terms included *regulation of signal transduction*, *signaling*, *cell population proliferation*, *multicellular organism development*, and *generation of neurons*. These processes were identified with high statistical significance (adjusted $p < 1e-7$) and strong enrichment scores ($-\log_{10}(p) > 7$), suggesting that *BRCA1* associated circRNA upregulation may be functionally linked to altered cell signalling and developmental pathways in HGSOC. The diversity of enriched terms reflects the pleiotropic roles of circRNAs in post-transcriptional regulation and supports their potential involvement in *BRCA1* driven tumour biology.

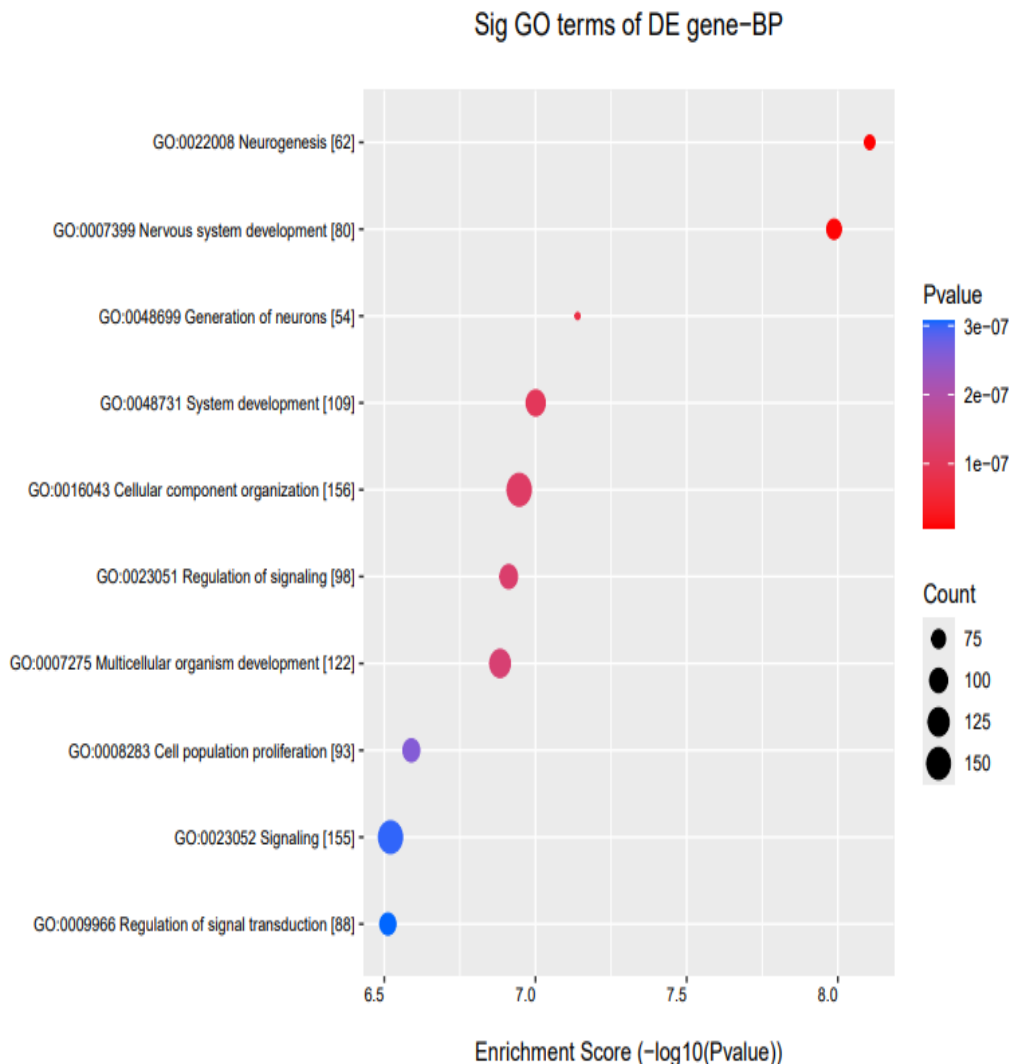


Figure 6-17 Top 10 enriched BPs in upregulated circRNAs (COV362 vs OVSAHO)

6.6.2 Cellular Components (CCs)

GO analysis of Cellular Component terms associated with upregulated circRNAs in COV362 identified significant enrichment in structures involved in secretion, intracellular transport, and membrane organisation (**Figure 6-18**). The most enriched terms included *extracellular exosome*, *extracellular vesicle*, *extracellular organelle*, *plasma membrane-bounded projections*, and components such as *cytoplasm*, *cytosol*, and *cell junctions*. These findings suggest that *BRCA1* associated circRNAs may play a role in exosome-mediated signalling and membrane-associated processes, potentially contributing to tumour cell communication or changes in subcellular architecture linked to *BRCA1* dysfunction.

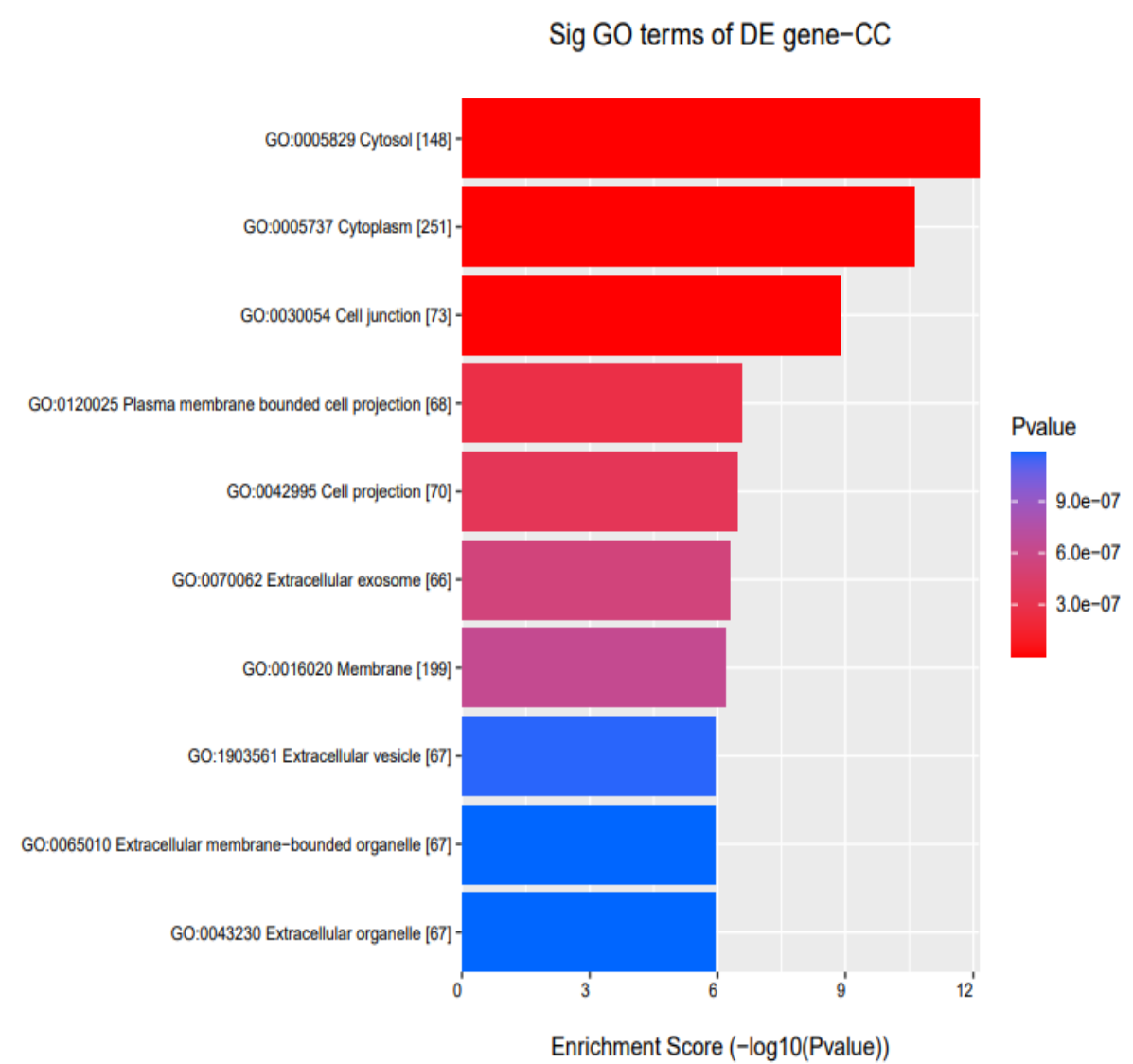


Figure 6-18 Top 10 enriched CCs in upregulated circRNAs (COV362 vs OVSAHO)

Bars represent $-\log_{10}(\text{p-value})$.

6.7 Molecular Function (MFs)

Analysis of Molecular Function GO terms revealed significant enrichment in enzymatic and binding activities among circRNAs upregulated in COV362 vs OVSAHO (Figure 6-19). The top enriched functions included *catalytic activity*, *protein binding*, *kinase activity*, *protein kinase activity*, and *enzyme regulator activity*. Additional terms such as *guanyl-nucleotide exchange factor activity* and *phosphotransferase activity* also featured prominently. These molecular functions suggest that *BRCA1*-linked circRNAs may influence intracellular signalling cascades, post-translational modification, and broader regulatory mechanisms, potentially contributing to altered cellular behaviour in *BRCA1*-mutant ovarian cancer cells.

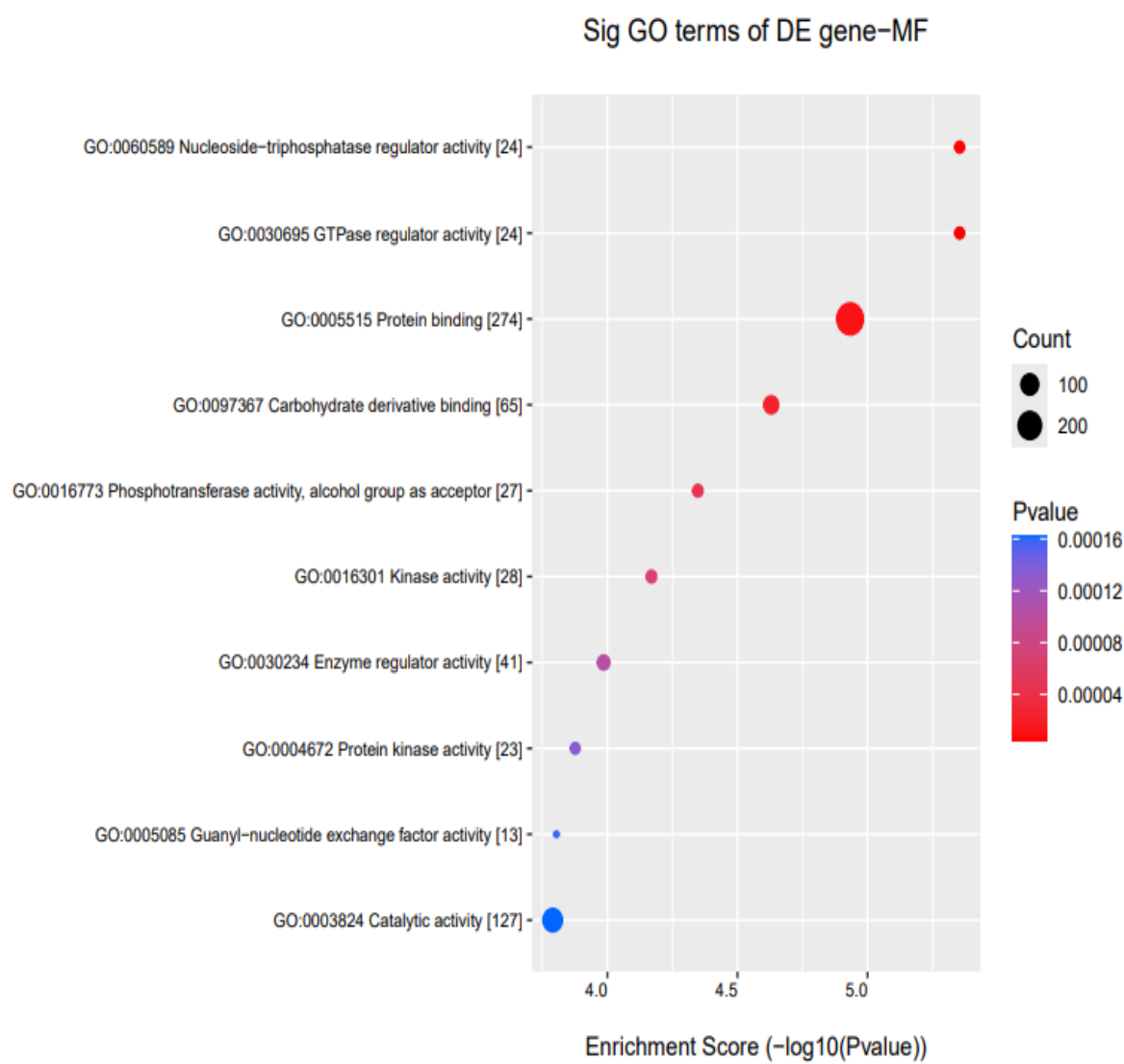


Figure 6-19 Top 10 enriched MF upregulated in circRNAs (COV362 vs OVSAHO)

6.7.1 GO Enrichment in KURAMOCHI vs OVSAHO

6.7.1.1 Biological Process (BPs)

GO enrichment analysis of circRNAs upregulated in KURAMOCHI (*BRCA2* mutant) compared to OVSAHO (*BRCA* wildtype) revealed significant enrichment of BPs related to organelle organisation, signalling regulation, and cytoskeletal dynamics (**Figure 6-20**). The top enriched GO terms included *regulation of signaling*, *macromolecule modification*, *cytoskeleton organization*, *regulation of organelle organization*, and *cellular localization*. These terms were identified with high statistical significance (adjusted $p < 5e-11$) and strong enrichment scores ($-\log_{10}(p) > 10$), suggesting that circRNA upregulation in *BRCA2*-mutant cells may influence intracellular architecture and spatial regulation of signalling pathways. The enrichment in structural and regulatory processes highlights the potential role of circRNAs in mediating cellular adaptation to *BRCA2* dysfunction.

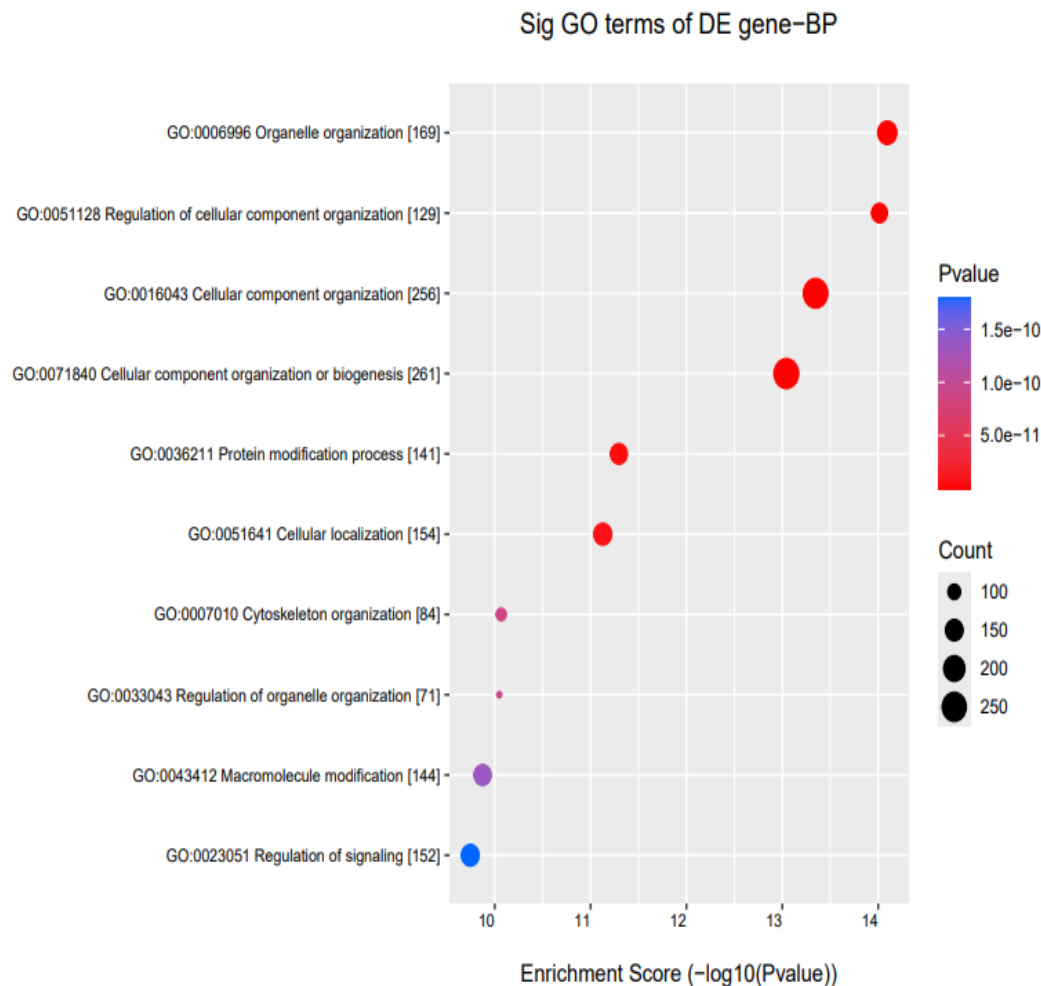


Figure 6-20 Top 10 enriched BPs in upregulated circRNAs (KURAMOCHI vs OVSAHO)

6.7.1.2 Cellular Components (CCs)

GO analysis of CCs terms associated with upregulated circRNAs in KURAMOCHI showed strong enrichment in structures involved in membrane dynamics and intracellular architecture (**Figure 6-21**). The top GO terms included *membrane-bounded organelle*, *focal adhesion*, *cell leading edge*, *nucleoplasm*, and *cytoplasm*. These enrichments suggest a prominent role for BRCA2-associated circRNAs in structural organisation of intracellular compartments, particularly in domains associated with polarity, adhesion, and spatial signalling. The results align with the BPenrichment, supporting a model in which BRCA2-linked circRNAs participate in reorganising the cellular landscape during oncogenic transformation.

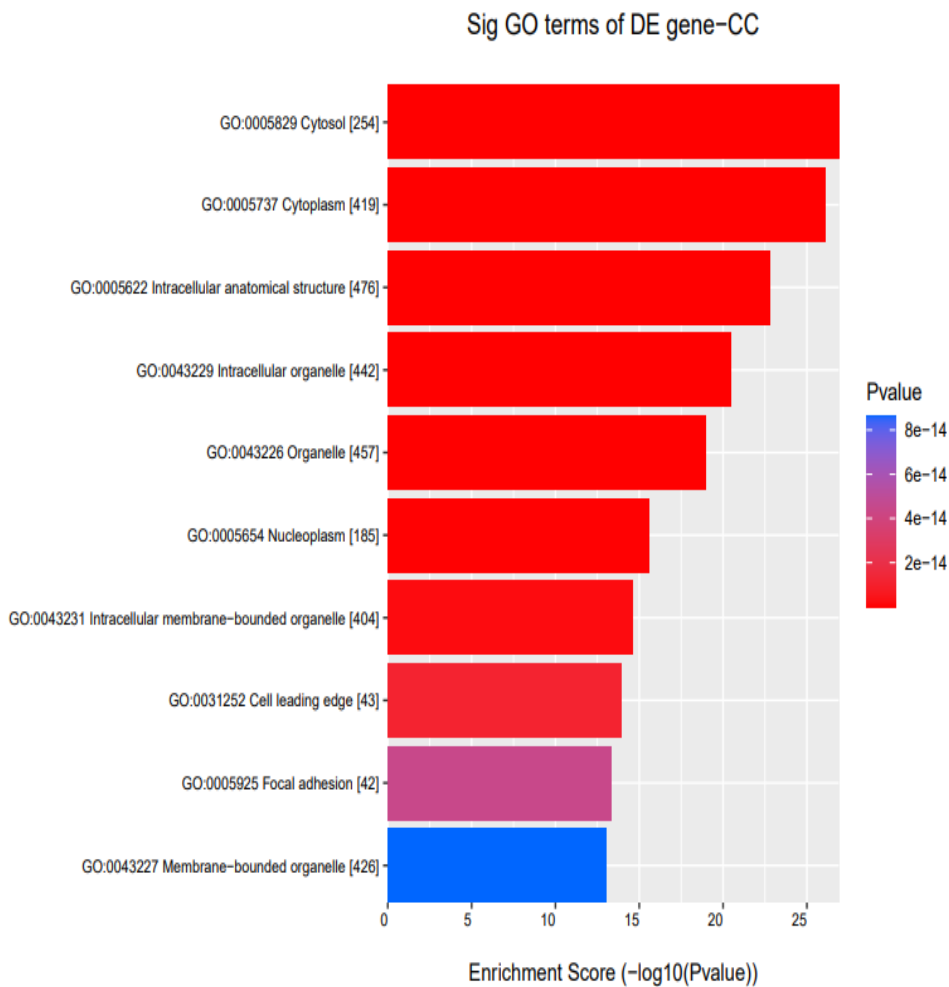


Figure 6-21 Top 10 enriched CCs in upregulated circRNAs (KURAMOCHI vs OVSAHO)

Bars represent $-\log_{10}(\text{p-value})$

6.7.1.3 Molecular Function (MF)

Analysis of MF GO terms for circRNAs upregulated in KURAMOCHI highlighted enrichment in various binding activities involved in nucleotide metabolism, protein interaction, and cell adhesion (Figure 6-22). Notable GO terms included *enzyme binding*, *purine nucleotide binding*, *nucleoside phosphate binding*, *cytoskeletal protein binding*, and *cadherin binding*. These results suggest that BRCA2-linked circRNAs may interact with key regulators of intracellular trafficking, signalling, and cytoskeletal control, pointing to potential mechanisms through which circRNAs contribute to BRCA2-related tumour biology.

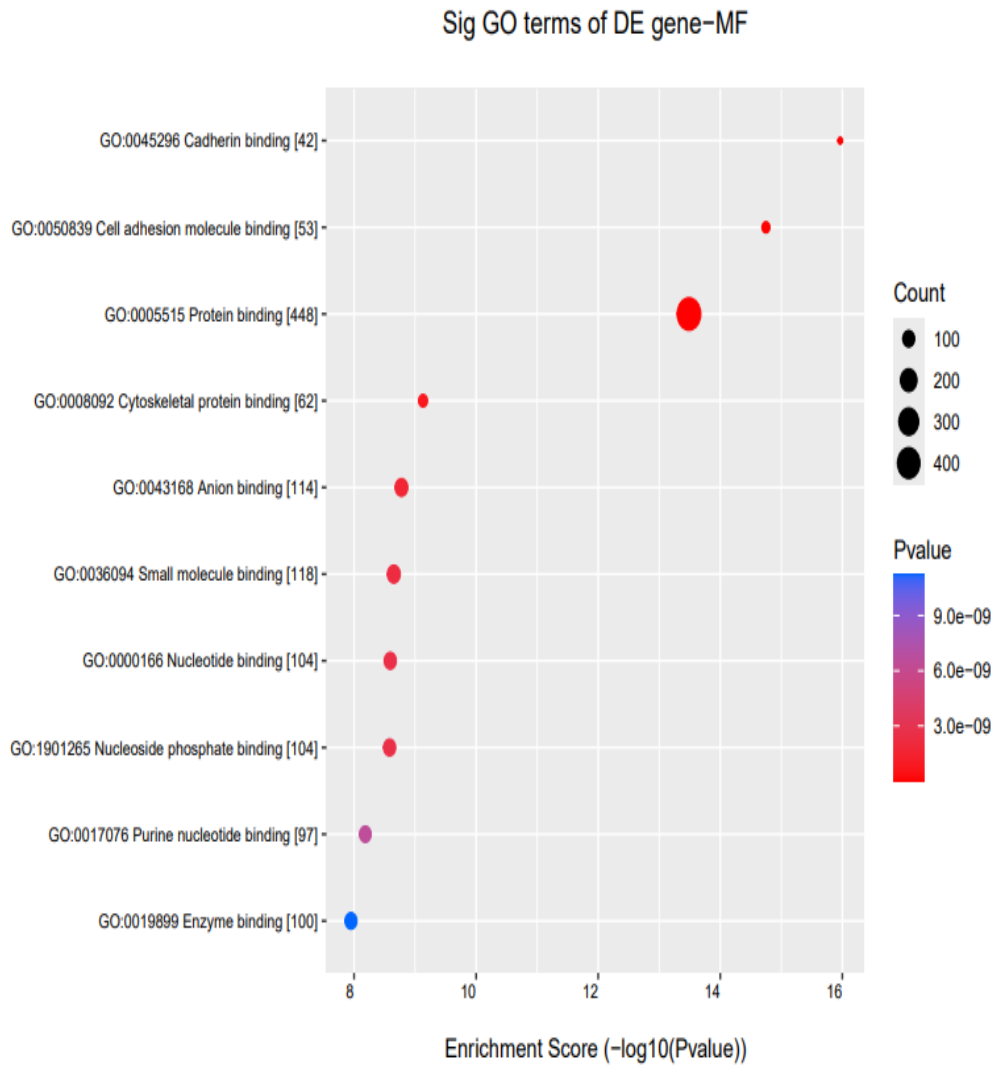


Figure 6-22 Top 10 enriched BPs in upregulated circRNAs (KURAMOCHI vs OVSAHO)

6.7.2 GO Enrichment in COV362 vs KURAMOCHI

6.7.2.1 Biological Process (BPs)

GO enrichment analysis of circRNAs upregulated in COV362 (BRCA1 mutant) compared to KURAMOCHI (BRCA2 mutant) revealed enrichment in BPs linked to cell organisation, development, and response to stimuli (Figure 6-23). Top GO terms included chromosome segregation, tissue morphogenesis, multicellular organism development, cell projection organization, and cellular response to stimulus. These findings suggest that circRNAs upregulated in BRCA1-mutant cells may be involved in the structural and developmental rewiring of cancer cells, including mitotic control and changes in cell shape or polarity. The enrichment of morphogenesis- and projection-related terms further supports a role for BRCA1-associated circRNAs in regulating cellular architecture and differentiation potential.

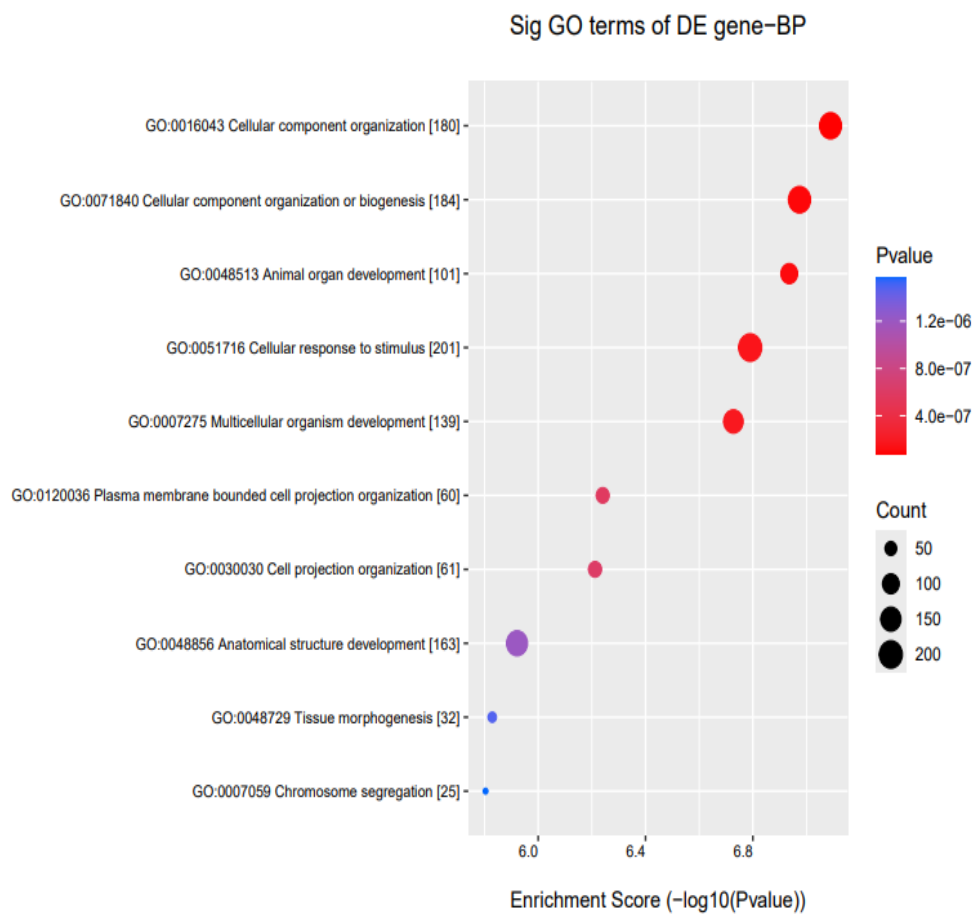


Figure 6-23 Top 10 enriched BPs in upregulated circRNAs (COV362 vs KURAMOCHI)

6.7.2.2 Cellular Components (CCs)

Analysis of CC GO terms highlighted enrichment in structures associated with cell projection, cytoskeleton, and extracellular communication (**Figure 6-24**). Highly ranked terms included cell projection, extracellular exosome, plasma membrane-bounded cell projection, cell junction, and cytoskeleton. The localisation of upregulated circRNAs to these components suggests that BRCA1-associated circRNAs may influence tumour behaviour through modulation of physical cell structures and intercellular signalling. This may reflect divergent cytoskeletal or migratory profiles between BRCA1- and BRCA2-mutant HGSOC models.

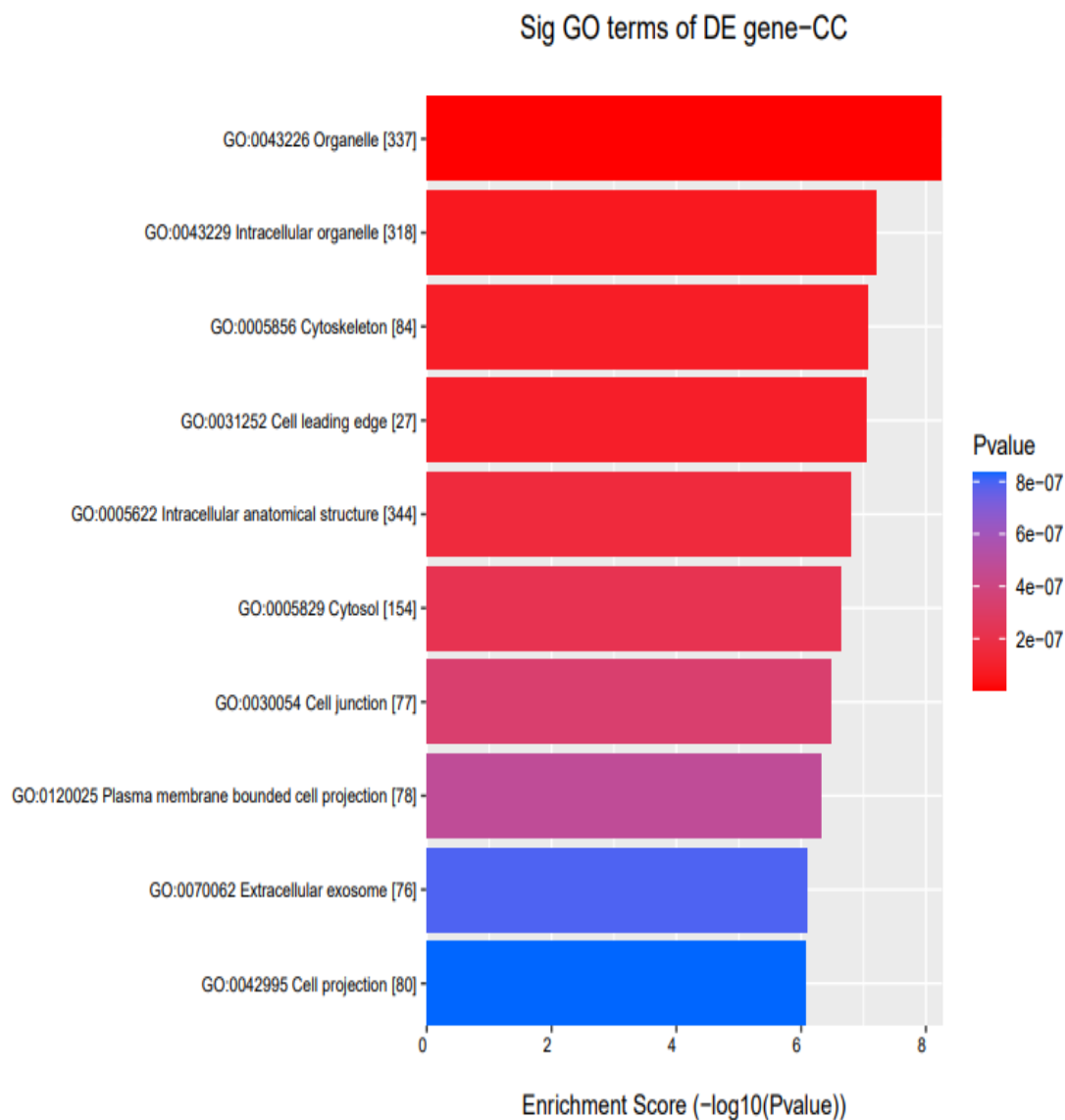


Figure 6-24 Top 10 enriched CCs in upregulated circRNAs (COV362 vs KURAMOCHI)

Bars represent $-\log_{10}(\text{p-value})$

6.7.2.3 Molecular Function (MF)

MF GO analysis revealed enrichment in diverse binding and catalytic activities among circRNAs upregulated in COV362 (Figure 6-25). The top enriched functions included carbohydrate derivative binding, catalytic activity acting on DNA, protein kinase activity, collagen binding, and cytoskeletal protein binding. These terms point toward a potential role for BRCA1-linked circRNAs in modulating DNA-associated enzymatic processes, kinase signalling, and extracellular matrix interaction. Compared to BRCA2-mutant cells, BRCA1-mutant cells may therefore exhibit a more transcriptionally dynamic and signal-responsive circRNA profile, particularly in pathways associated with cell structure and genomic regulation.

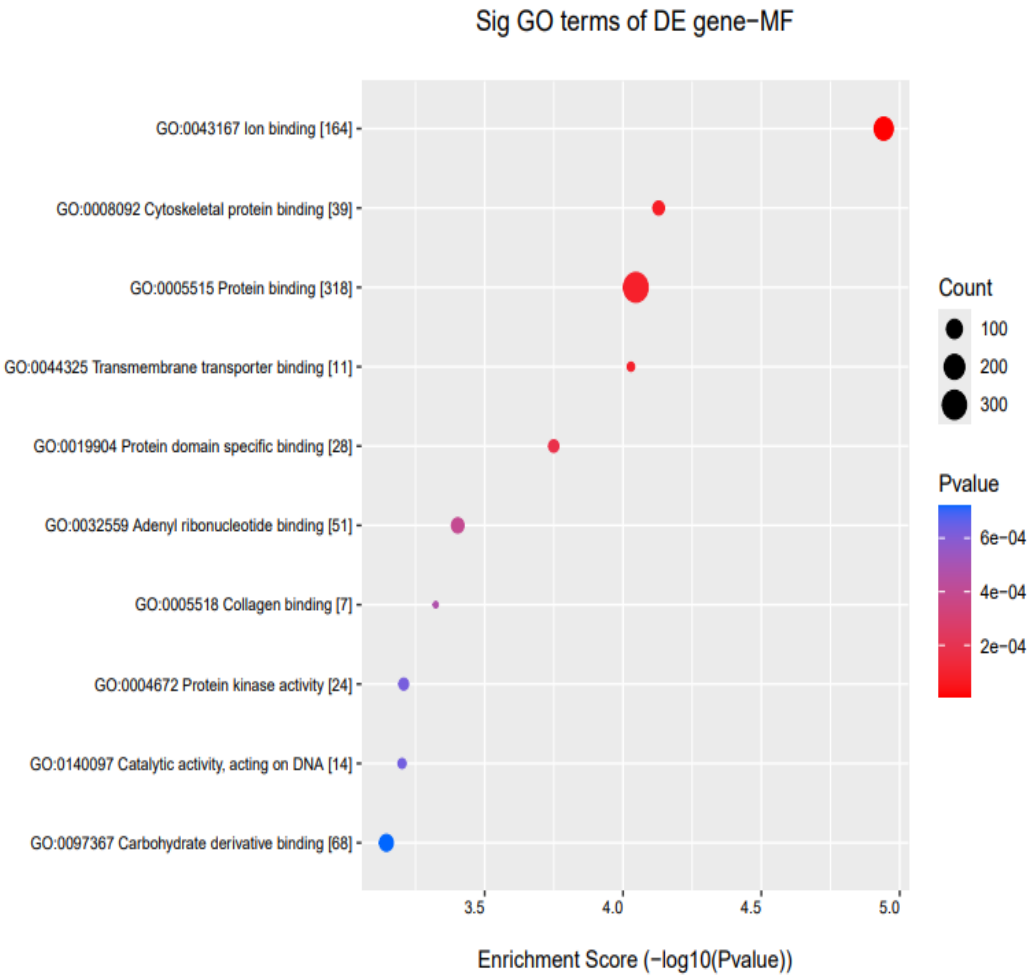


Figure 6-25 Top 10 enriched MFs in upregulated circRNAs (COV362 vs KURAMOCHI)

6.7.3 Pathways Enriched in *BRCA* Linked circRNAs

To further explore the biological relevance of circRNAs upregulated in *BRCA* related OC, KEGG pathway enrichment analysis was performed across key pairwise comparisons. This approach maps the host genes of differentially expressed circRNAs to established signalling and metabolic pathways, giving insight into the broader cellular processes they may influence. As with the GO analysis, the focus was placed on upregulated circRNAs, given their greater stability and experimental tractability. Enriched pathways were identified based on statistical overrepresentation, highlighting functional networks potentially regulated by *BRCA* associated circRNA expression. It is acknowledged that while many enriched pathways such as glioma or EGFR signalling are named for specific disease contexts, they represent broader oncogenic signalling cascades that may be relevant across multiple tumour types, including OC.

6.7.4 KEGG Pathway Enrichment in COV362 vs OVSAHO

Pathway analysis of upregulated circRNAs in COV362 compared to OVSAHO identified multiple significantly enriched KEGG pathways, primarily related to cell signalling, cancer biology, and cytoskeletal regulation (**Figure 6-26**). The most enriched pathways included regulation of the *actin cytoskeleton*, *choline metabolism in cancer*, *thyroid hormone signalling*, *neurotrophin signalling*, *ErbB signalling*, and *focal adhesion*. Cancer-specific pathways were also identified, such as *glioma*, *non-small cell lung cancer*, *hepatocellular carcinoma*, and *EGFR tyrosine kinase inhibitor resistance*. **Figure 6-26** presents enrichment scores as $-\log_{10}(\text{p-values})$, reflecting the statistical significance of pathway *over-representation*. Pathways with higher scores are more significantly enriched. Importantly, these scores are not solely determined by the number of genes involved. For example, the glioma pathway had a high enrichment score ($-\log_{10}p > 4$) despite involving only nine upregulated circRNA-associated genes. This indicates that these genes are over-represented relative to what would be expected, even if the absolute number is modest.

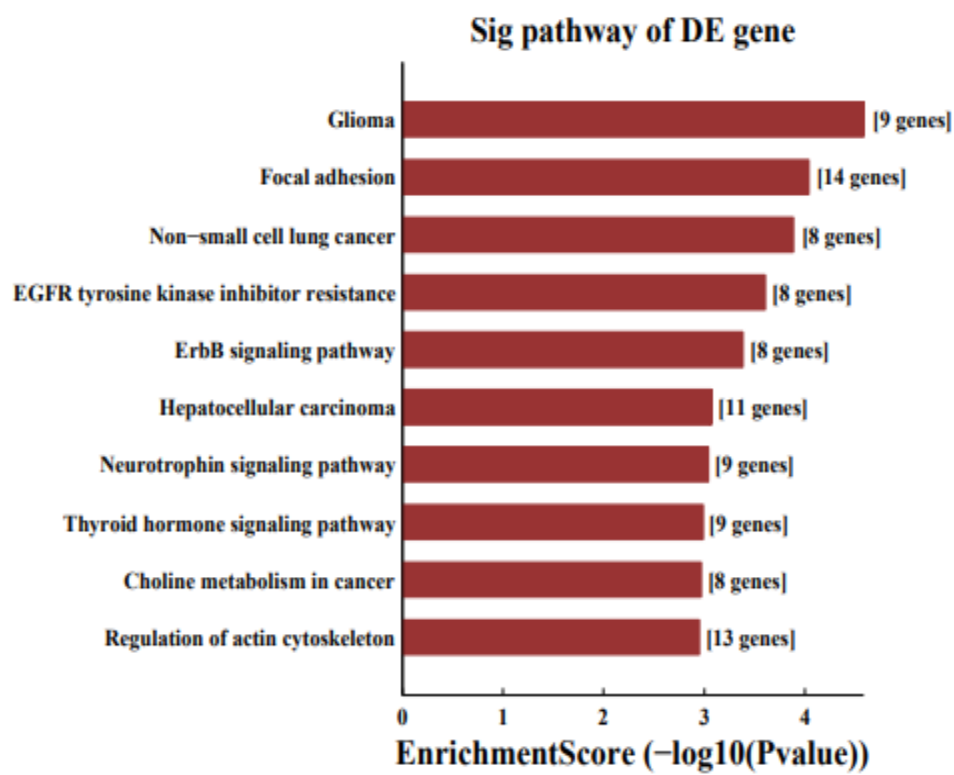


Figure 6-26 KEGG pathway of upregulated circRNAs (COV362 vs OVSAHO)

Bar plot shows the top 10 pathways ranked by enrichment score ($-\log_{10} \text{p-value}$)

6.7.5 KEGG Pathway Enrichment in KURAMOCHI vs OVSAHO

Pathway analysis of circRNAs upregulated in KURAMOCHI compared to OVSAHO revealed significant enrichment in pathways related to cancer signalling, cytoskeletal regulation, and cellular stress responses (**Figure 6-27**). The top enriched KEGG pathways included regulation of actin cytoskeleton, proteoglycans in cancer, focal adhesion, *EGFR* tyrosine kinase inhibitor resistance, Fc gamma R-mediated phagocytosis, and ErbB signalling pathway. Additional pathways of interest included AGE–RAGE signalling in diabetic complications, renal cell carcinoma, non–small cell lung cancer, and Parkinson disease. Several pathways had strong enrichment scores ($-\log_{10} p > 6$) and high gene counts, including 26 genes associated with proteoglycans in cancer and 25 with regulation of actin cytoskeleton.

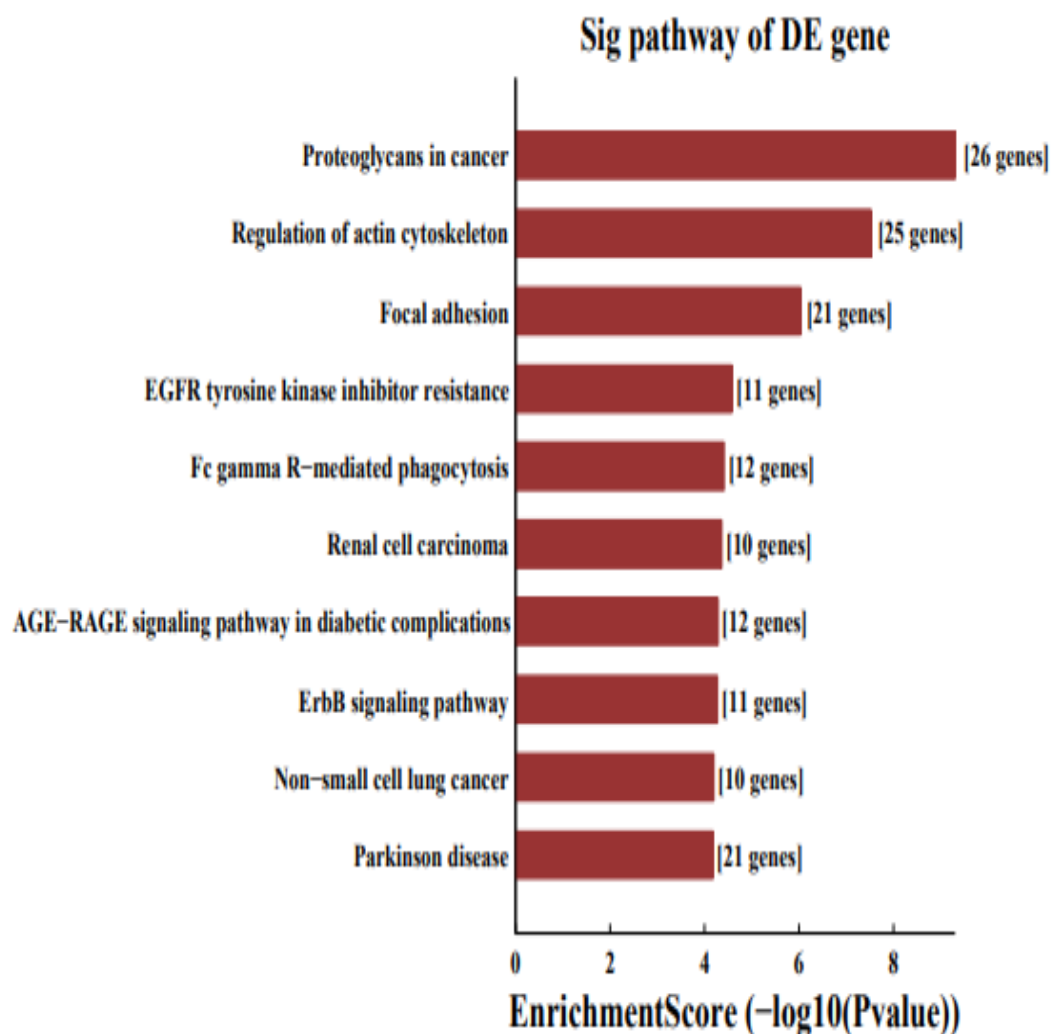


Figure 6-27 KEGG pathway of upregulated circRNAs (KURAMOCHI vs OVSAHO)

Bar plot shows the top 10 pathways ranked by enrichment score ($-\log_{10} p$ -value)

6.7.6 KEGG Pathway Enrichment in COV362 vs KURAMOCHI

Pathway analysis of circRNAs upregulated in COV362 compared to KURAMOCHI revealed significant enrichment in pathways related to cell cycle regulation, DNA metabolism, and oncogenic signalling (Figure 6-28) The most enriched KEGG pathways included DNA replication, cell cycle, mismatch repair, glioma, and ErbB signalling. Additional pathways of interest included thyroid hormone signalling, neurotrophin signalling, and EGFR tyrosine kinase inhibitor resistance. Enrichment scores exceeded $-\log_{10} p$ of 4.5 for several terms, with high gene representation, including ten genes in *DNA replication* and nine in *glioma*. These results suggest that *BRCA1* linked circRNAs may contribute to transcriptional regulation, proliferative signalling, and cell cycle control in a manner distinct from *BRCA2* mutant cells. The prominence of DNA damage response and replication-associated pathways aligns with known roles of *BRCA1* in maintaining genomic integrity and further supports the functional relevance of circRNAs in *BRCA* related tumour biology.

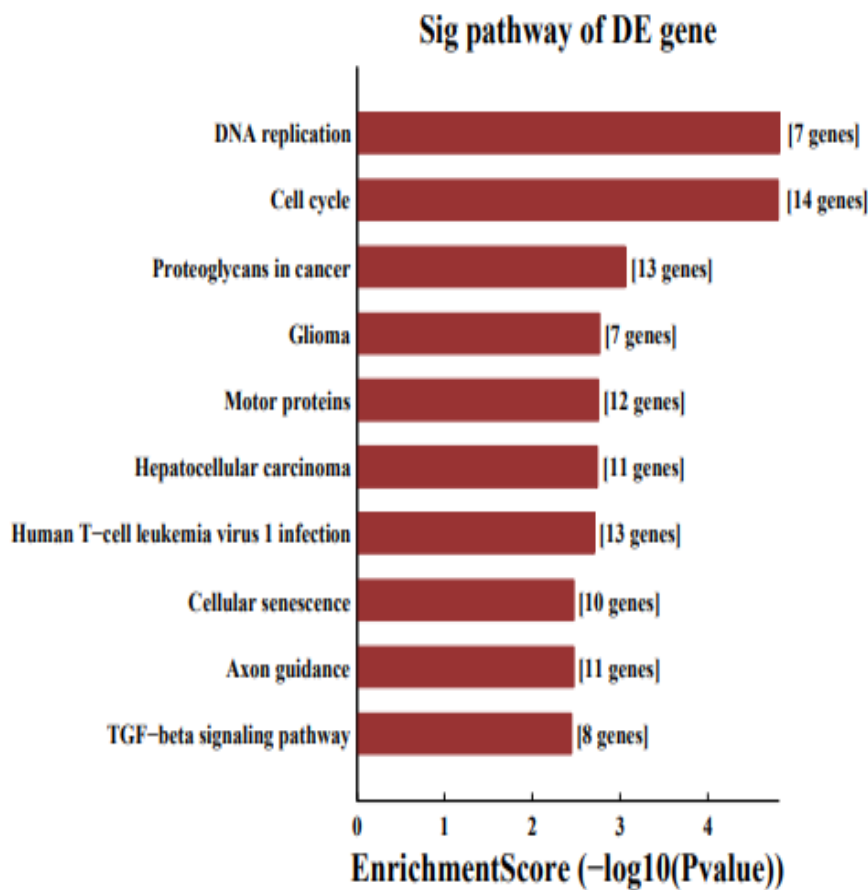


Figure 6-28 KEGG pathway of upregulated circRNAs (COV362 vs KURAMOCHI)

Bar plot shows the top 10 pathways ranked by enrichment score ($-\log_{10} p\text{-value}$)

6.7.7 Chromosomal Distribution of Differentially Expressed circRNAs

To further investigate the genomic origin of differentially expressed circRNAs in BRCA-associated OC, circRNA host genes were mapped across the human genome by chromosome. This analysis provides insight into the chromosomal landscape of circRNA deregulation and may help identify genomic hotspots or structural biases in circRNA biogenesis associated with *BRCA1* and *BRCA2* mutation status. In the COV362 (*BRCA1* mutant) vs OVSAHO (wildtype) comparison, 508 circRNAs were upregulated and 362 were downregulated, with high representation on chromosomes 1, 2, and 17 (Figure 6-29).

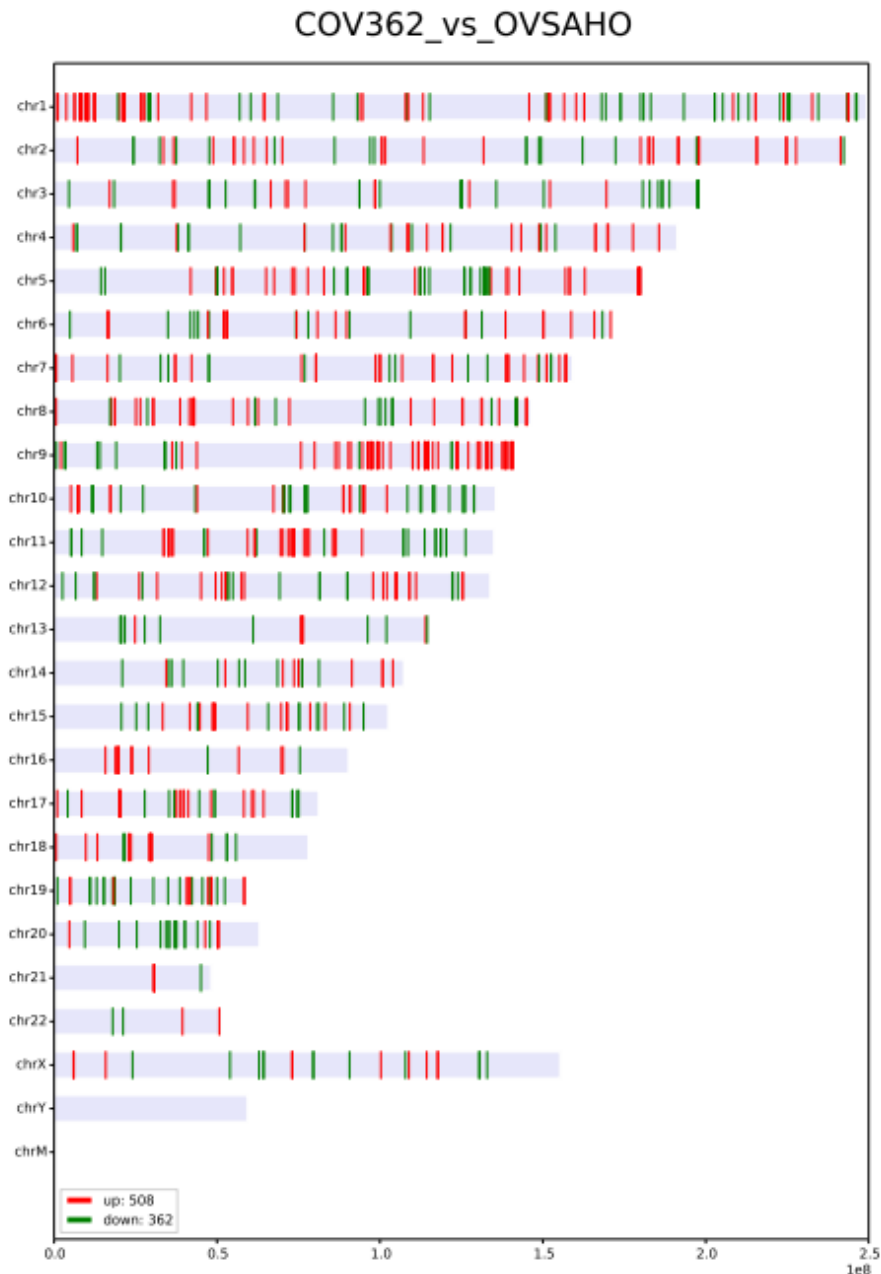


Figure 6-29 Chromosomal distribution of circRNAs in COV362 vs OVSAHO

In the KURAMOCHI (*BRCA2* mutant) vs OVSAHO comparison, 876 circRNAs were upregulated and 675 downregulated, again showing strong contribution from gene-dense chromosomes including 1, 2, and 17 (**Figure 6-30**).

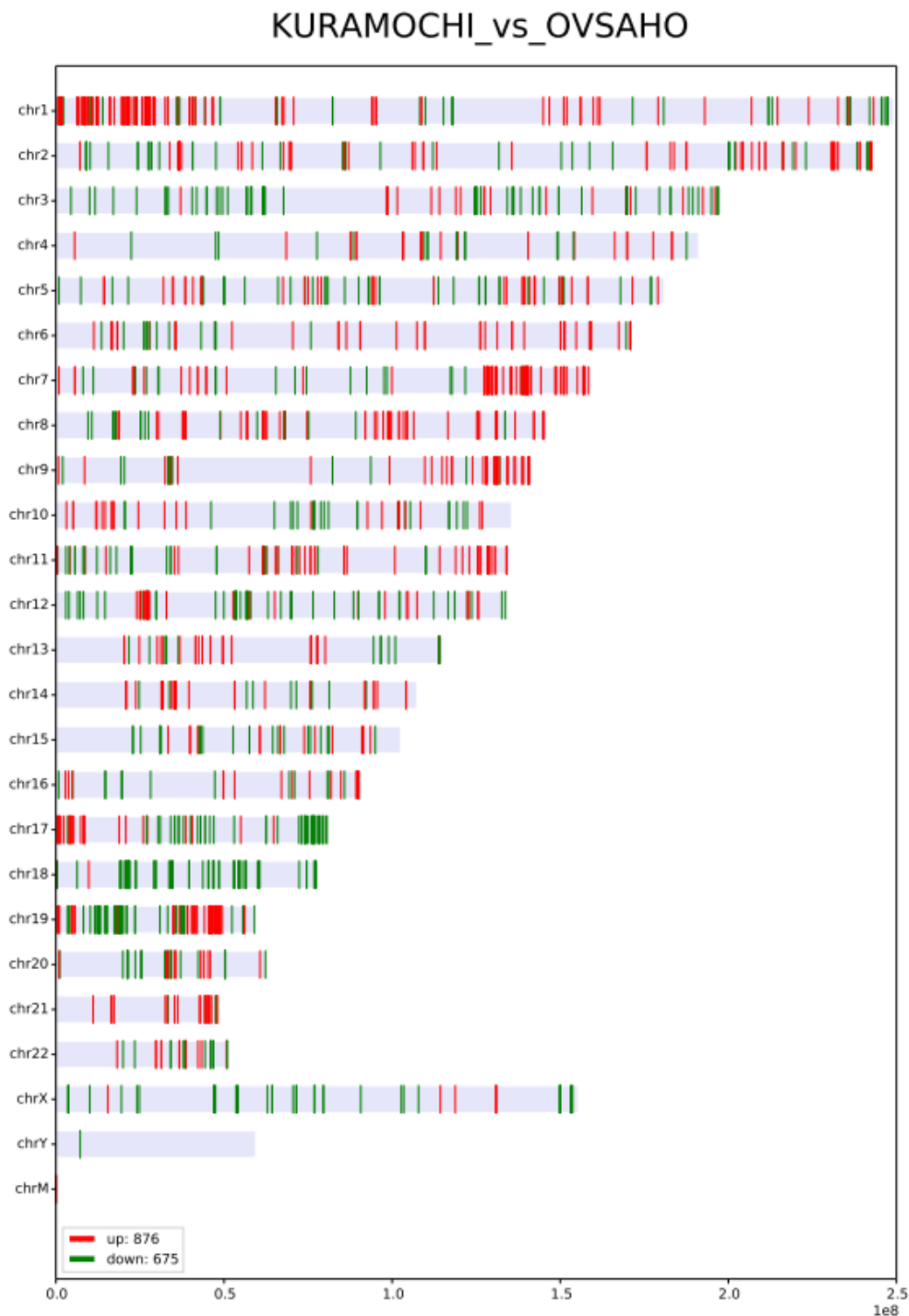


Figure 6-30 Chromosomal distribution of circRNAs in KURAMOCHI vs OVSAHO

In the *BRCA1* vs *BRCA2* comparison (COV362 vs KURAMOCHI), 602 circRNAs were upregulated and 634 downregulated, with a similar distribution pattern (**Figure 6-31**).

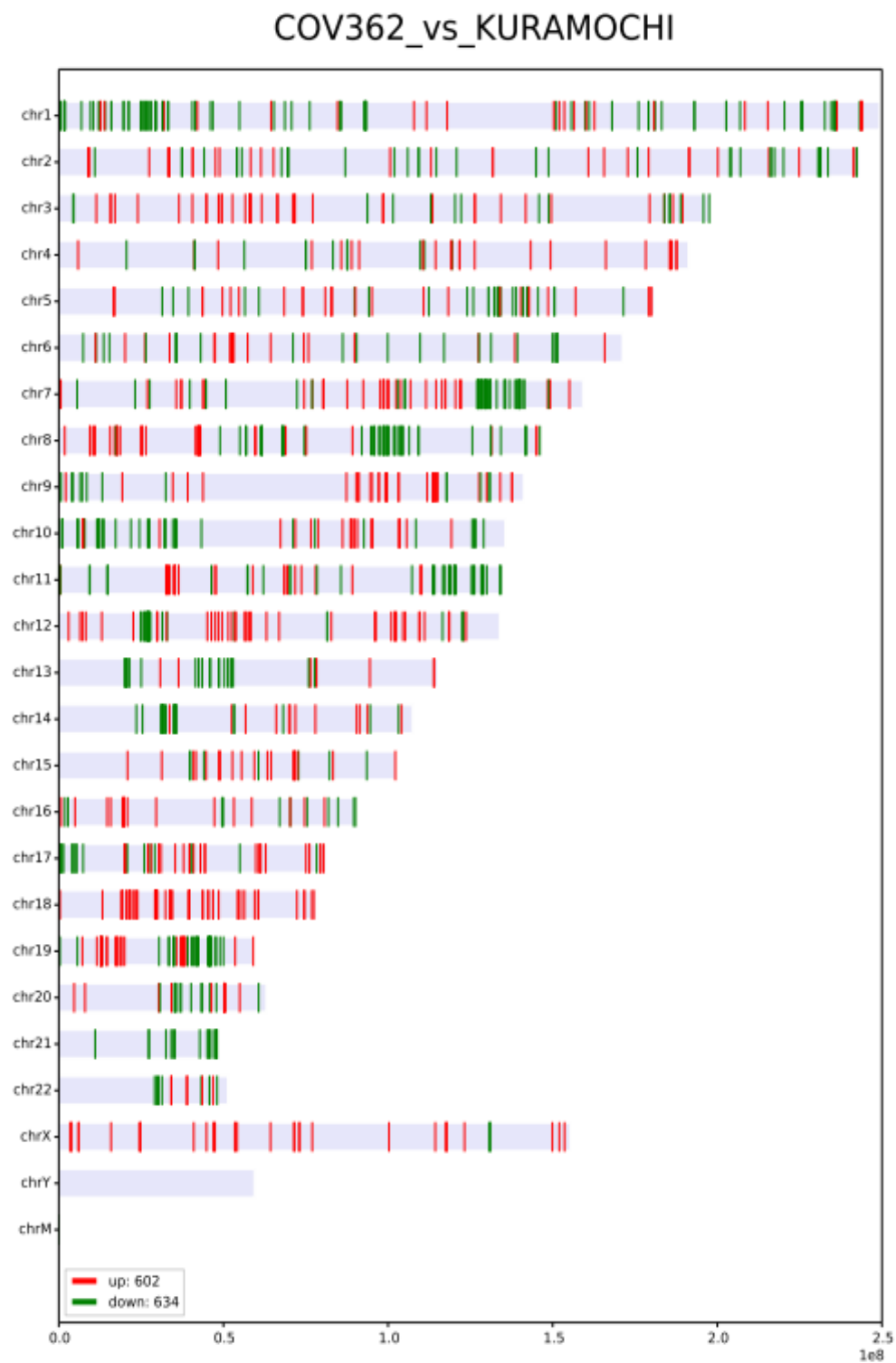


Figure 6-31 Chromosomal distribution circRNAs in COV362 vs KURAMOCHI

6.7.8 Differentially expressed circRNA profiling of OC cell lines

CircRNA expression levels were analysed across cell lines stratified by *BRCA* status, with each cell line assessed in biological triplicate. Differentially expressed circRNAs were identified by calculating fold change (FC), defined as the ratio of group averages, between *BRCA1*-mutated vs. *BRCA* wildtype cell lines, and *BRCA2*-mutated vs. *BRCA* wildtype cell lines. Statistical significance was determined using a paired Student’s *t*-test, and p-values were adjusted using the false discovery rate (FDR), with a significance threshold of $FDR < 0.05$. CircRNAs with an $FC \geq 2.0$ and a p-value < 0.05 were considered significantly differentially expressed between *BRCA* mutant and wildtype cell lines. The results, displayed in **Figure 6-32** below, show the overlap of significantly upregulated circRNAs across the three pairwise comparisons of *BRCA*-stratified OC cell lines.

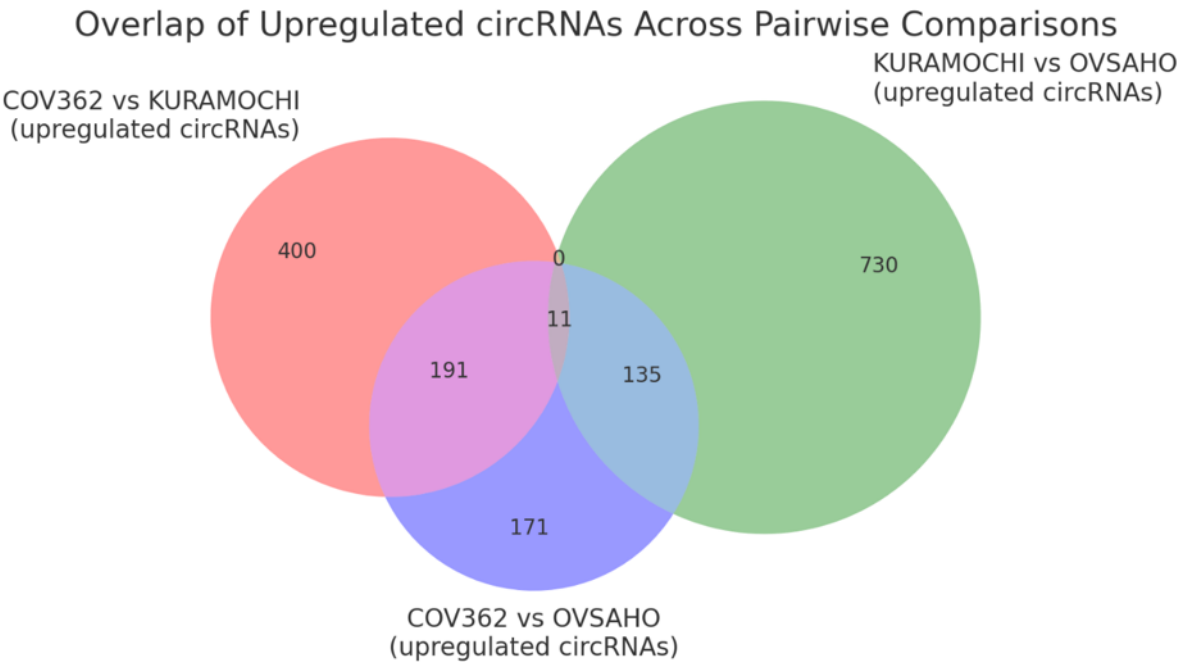


Figure 6-32 Overlap of Upregulated circRNAs Across Pairwise Comparisons

Created by the author with assistance from ChatGPT (OpenAI, 2025)

The top five upregulated circRNAs were selected for both the KURAMOCHI vs. OVSAHO comparison (**Table 6-3**) and the COV362 vs. OVSAHO comparison (**Table 6-4**). Additionally, the top six circRNAs commonly upregulated in both comparisons were identified (**Table 6-5**). All selected circRNAs were exonic in origin.

circRNA	P-value, FDR and FC			Annotations				Group--Raw Intensity			
	P-value	FDR	FC (abs)	Alias	source	chrom	GeneSymbol	KURAMOCHI (raw)	OVSAHO (raw)	KURAMOCHI (normalized)	OVSAHO (normalized)
hsa_circRNA_400223	<0.01	0.02	109.74		25242744 ³	chr1	UQCRH	7330.50	82.50	13.30	6.53
hsa_circRNA_061800	0.01	0.04	24.26	hsa_circ_0061800	circBase	chr21	MX1	2778.25	108.00	11.57	6.97
hsa_circRNA_025636	<0.01	0.03	21.88	hsa_circ_0025636	circBase	chr12	RASSF8	7912.00	435.00	13.42	8.97
hsa_circRNA_104925	<0.01	0.01	21.23	hsa_circ_0088681	circBase	chr9	FAM129B	758.75	31.00	9.50	5.09
hsa_circRNA_088194	0.01	0.06	18.22	hsa_circ_0088194	circBase	chr9	TNC	1310.50	56.50	10.16	5.97

Table 6-3 Top 5 Upregulated circRNA KURAMOCHI vs. OVSAHO

circRNA	P-value, FDR and FC			Annotations				Group--Raw Intensity			
	P-value	FDR	FC (abs)	Alias	source	chrom	GeneSymbol	COV362 (raw)	OVSAHO(raw)	COV362 (normalized)	OVSAHO (normalized)
hsa_circRNA_021732	0.02	0.18	34.01	hsa_circ_0021732	circBase	chr11	CD44	4270.75	99.50	11.65	6.56
hsa_circRNA_400223	0.00	0.15	25.50		25242744 ²	chr1	UQCRH	3569.50	110.00	11.52	6.85
hsa_circRNA_100059	0.01	0.15	25.03	hsa_circ_0010020	circBase	chr1	DHRS3	2366.50	79.50	11.03	6.39
hsa_circRNA_102787	<0.01	0.15	16.52	hsa_circ_0001055	circBase	chr2	AFF3	1245.00	66.50	10.18	6.13
hsa_circRNA_104925	0.01	0.15	15.53	hsa_circ_0088681	circBase	chr9	FAM129B	634.00	37.50	9.25	5.30

Table 6-4 Top 5 Upregulated circRNA COV362 vs. OVSAHO

³ 25242744 refers to PMID 25242744 430. Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L. Complementary sequence-mediated exon circularization. Cell. 2014;159(1):134-47.

circRNA	Annotationa				P-value, Fold change and Regulation									
					KURAMOCHI vs.OVSAHO			COV362 vs.OVSAHO			COV362 vs.KURAMOCHI			
	Alias	source	chrom	GeneSymbol	P-value	FDR	FC (abs)	P-value	FDR	FC (abs)	P-value	FDR	FC (abs)	Regulation
hsa_circRNA_400223		25242744	chr1	UQCRH	<0.01	0.02	109.74	0.00	0.15	25.50	0.02	0.18	3.43	down
hsa_circRNA_017873	hsa_circ_0017873	circBase	chr10	VIM	<0.01	0.01	17.83	0.01	0.15	6.72	0.02	0.19	2.31	down
hsa_circRNA_021732	hsa_circ_0021732	circBase	chr11	CD44	<0.01	0.02	9.30	0.02	0.18	34.01	0.01	0.15	6.00	up
hsa_circRNA_007919	hsa_circ_0007919	circBase	chr17	ABR	<0.01	0.01	8.11	0.01	0.15	3.54	0.01	0.17	2.40	down
hsa_circRNA_003300	hsa_circ_0003300	circBase	chrX	LRCH2	<0.01	0.00	3.32	0.00	0.14	7.70	<0.01	0.13	2.51	up
hsa_circRNA_008026	hsa_circ_0008026	circBase	chr4	CAMK2D	0.01	0.04	3.07	0.00	0.15	6.39	0.03	0.21	2.10	up

Table 6-5 Top 6 Common upregulated circRNAs

6.7.9 Validation of circRNAs in cell lines

6.7.10 Selection, Design, and Optimization of circRNAs

Custom-designed outward-facing primers were used to validate selected circRNAs identified as upregulated in *BRCA*-mutant OC cell lines (**Appendix 8**). Five circRNAs upregulated in COV362 (*BRCA1* mutant) vs. OVSAHO (*BRCA* wildtype) and KURAMOCHI (*BRCA2* mutant) vs. OVSAHO (*BRCA* wildtype) comparisons were selected for validation by qPCR. An additional six circRNAs that were commonly upregulated (labelled as 'Common' in group in **Table 6-6**) in both *BRCA1* mutant and *BRCA2* mutant lines (COV362 and KURAMOCHI) relative to wildtype (OVSAHO) were also included, bringing the total number of candidates to 14. Two circRNAs overlapped between the "up in individual mutant" and "common" groups, resulting in 12 unique circRNAs evaluated using qPCR. Primer pairs were designed to span the backsplice junction, and conditions were optimised using both endpoint PCR and qPCR across all three cell lines.

No.	circRNA	Group	Left Primer	Right Primer
1	hsa_circRNA_400223	Upregulated in BRCA-mutants (vs. WT)	GGGAGCGGCTAGAGCTCTGT	CGAGGGACCATTGCATAGGA
2	hsa_circRNA_017873	Upregulated in BRCA-mutants (vs. WT)	TTCCAGCAAGTATCCAACCA	CCTCTTCGTGGAGTTTCTTCA
3	hsa_circRNA_0021732	Upregulated in BRCA-mutants (vs. WT)	AAGGTGGAGCAAACACAACC	GATGCTCATGGTGAATGAGG
4	hsa_circRNA_007919	Upregulated in BRCA-mutants (vs. WT)	GACAAGATCATGGGCAAAGG	AGGGCTTCCAGCTGGTTAAT
5	hsa_circRNA_003300	Upregulated in BRCA-mutants (vs. WT)	CATGTACCATCACCAGCAGTG	CATTGCCACTTGAACCTCTCC
6	hsa_circRNA_008026	Upregulated in BRCA-mutants (vs. WT)	CCTTATGGAAAGCCAGTGGA	GCAAATTCTCAGGCTTCAGG
7	hsa_circRNA_021732	Up regulated in COV362 vs. OVSAHO	AAGGTGGAGCAAACACAACC	GATGCTCATGGTGAATGAGG
8	hsa_circRNA_100059	Up regulated in COV362 (BRCA2) vs. OVSAHO (WT)	ACAATGCATGCCCTCGTTAT	TGATGTGTTGGGACTTGAGG
9	hsa_circRNA_102787	Up regulated in COV362 (BRCA2) vs. OVSAHO(WT)	GCTCTCCAAGTTCAGCATCC	TTAAACGTGCCATCATCCTG
10	hsa_circRNA_104925	Up regulated in COV362 (BRCA2) vs. OVSAHO (WT); Up in KURA vs.	CATCCTTATGCGCGTCACT	GTCAGGATCTTCCCGGTTTT

		OVSCHO		
11	hsa_circRNA_026359	Up regulated in COV362 (<i>BRCA2</i>) vs. OVSCHO (WT)	CCTGGTCCCAAGACAGTGAG	GGTCTCCAGCAGCTTGTCT
12	hsa_circRNA_061800	Up regulated in KURA (<i>BRCA1</i>) vs. OVSCHO (WT)	CCCAGTGTTTTAGGAGCATGA	CGGATCAGCTTCTCACCTTC
13	hsa_circRNA_025636	Up regulated in KURA (<i>BRCA1</i>) vs. OVSCHO (WT)	GGTGGATGGAGTTCAGAGGA	AGTCTCCGAGCCAGTGAGAA
14	hsa_circRNA_088194	Up regulated in KURA (<i>BRCA1</i>) vs. OVSCHO (WT)	CTGCCACGGAATACACACTG	TTGTCTGAAGACCCCTTCATC

Table 6-6 Left and right primers for selected upregulated circRNA

*WT = wildtype

6.7.11 Primers Selected for BRCA mutant specific

After selecting primers, they were optimized using end-point PCR. The selected candidates for further validation included hsa_circ_400223, hsa_circ_008026, and hsa_circ_003300, all of which were commonly upregulated across all three cell lines. Additionally, hsa_circ_0025636 showed increased expression specifically in Kuramochi (*BRCA2* mutant) compared to OVSAHO (*BRCA* wild type). hsa_circ_0100059, which was upregulated in COV362 (*BRCA1* mutated) relative to OVSAHO, was noted as specific for *BRCA1* mutations. Quality of cDNA was confirmed using 18s primer.

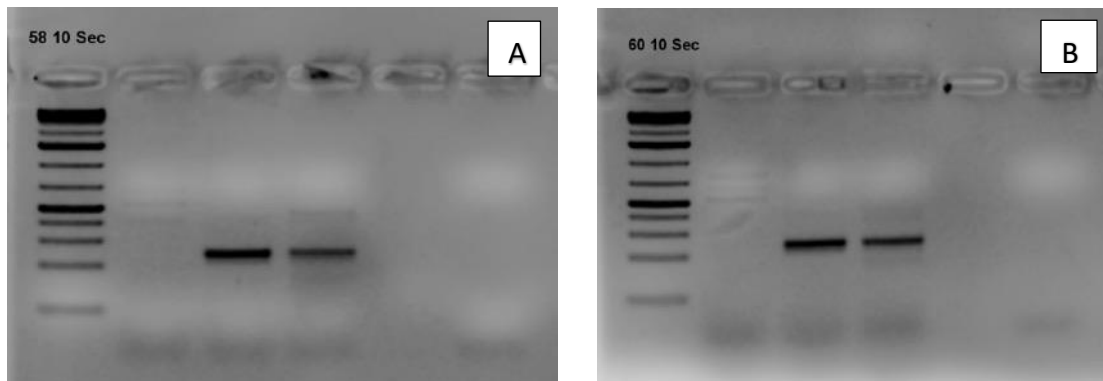


Figure 6-33 (A) hsa_circ_400223 forward primer, (B) hsa_circ_400223 reverse primer

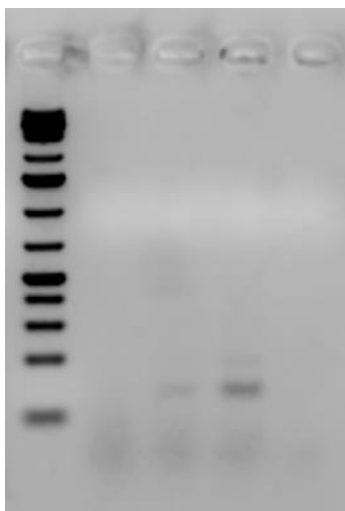


Figure 6-34 hsa_circ_008026

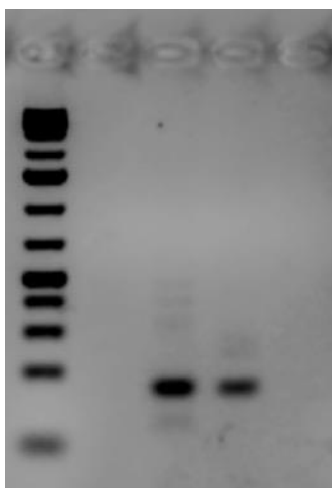


Figure 6-35 hsa_circ_0025636

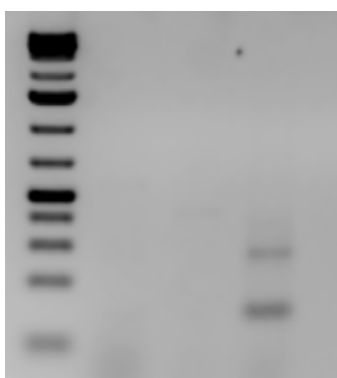


Figure 6-36 hsa_circ_0100059

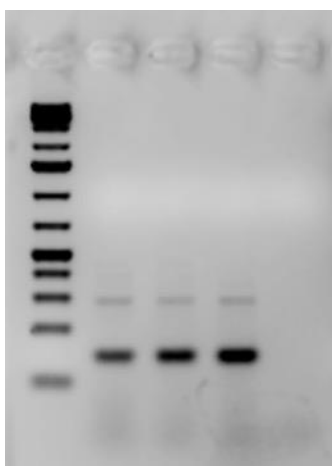


Figure 6-37 hsa_circ_003300

Overall, three circRNAs (*hsa_circ_400223*, *hsa_circ_008026*, and *hsa_circ_003300*) were identified as consistently upregulated across all OC cell lines, while *hsa_circ_0025636* and *hsa_circ_0100059* demonstrated *BRCA2*- and *BRCA1*-specific expression patterns, respectively. These findings guided the selection of five circRNAs for further validation across *BRCA*-mutant and wildtype OC models. The consistently upregulated circRNAs were prioritised to confirm reproducibility of expression trends, while those showing *BRCA*-specific enrichment were selected to explore potential genotype-associated expression patterns. The selected circRNAs, their expression profiles, and validation rationale are summarised in **Table 6-7**.

circRNA ID	Expression Pattern	<i>BRCA</i> Context	Validation Rationale
hsa_circ_400223	Upregulated in all three OC cell lines	<i>BRCA1</i> (COV362), <i>BRCA2</i> (KURAMOCHI), WT (OVSAHO)	Consistently upregulated across all models
hsa_circ_008026	Upregulated in all three OC cell lines	<i>BRCA1</i> , <i>BRCA2</i> , WT	Consistently upregulated across all models
hsa_circ_003300	Upregulated in all three OC cell lines	<i>BRCA1</i> , <i>BRCA2</i> , WT	Consistently upregulated across all models
hsa_circ_0025636	Upregulated in KURAMOCHI vs. OVSAHO	<i>BRCA2</i> -mutant specific	Increased expression in <i>BRCA2</i> -mutant cell line
hsa_circ_0100059	Upregulated in COV362 vs. OVSAHO	<i>BRCA1</i> -mutant specific	Increased expression in <i>BRCA1</i> -mutant cell line

Table 6-7 circRNAs selected for validation across *BRCA* statuses

6.7.12 Primers Selected for *BRCA* wildtype specific

circRNA primers specific for the *BRCA* wildtype were designed, identified as being downregulated in KURA and COV362 compared to OVSAHO. The objective was to validate these findings; however, the primers did not perform as expected, exhibiting unreliable amplification during endpoint PCR (**Figure 6-38**), which hindered further validation of the results and this avenue was abandoned.

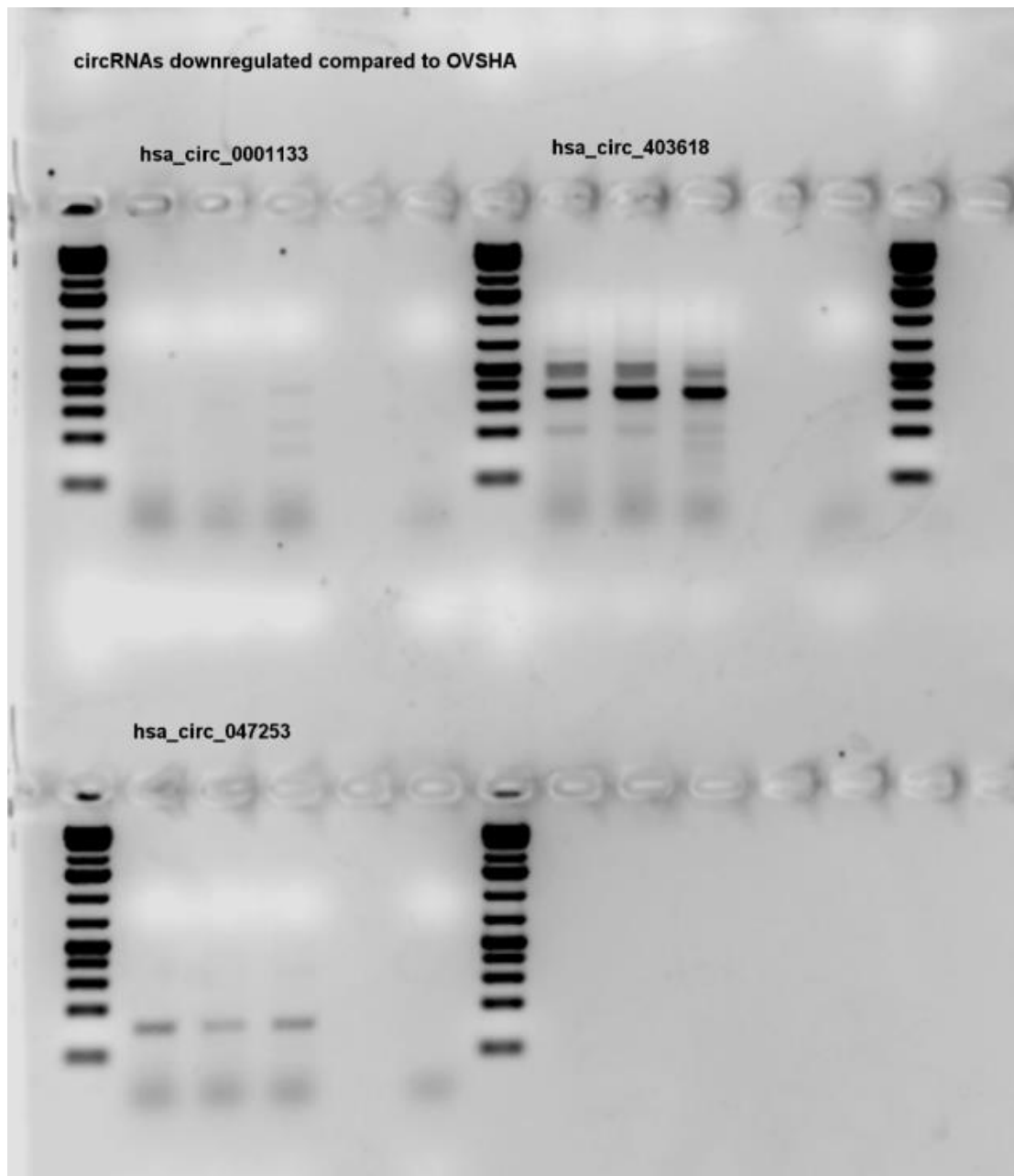


Figure 6-38 Endpoint PCR demonstrating nonspecific amplification in *BRCA* WT

6.7.13 Cell Line Validation of circRNAs Using RT-PCR

After selecting circRNA of interest and optimisation and validating primers, 5 circRNA were chosen to bring forward for further validation with qPCR, the results of which are displayed in **Table 6-8**. In this analysis of circRNA expression across COV362 (*BRCA1*) and KURAMOCHI (*BRCA2*) mutant and OVSAHO (wildtype) OC cell lines, several circRNAs showed distinctive expression patterns as outlined in **Figure 6-39**. Experiments were performed in technical triplicate. Of note, hsa_circRNA_100059 displayed the darkest, most defined bands in the *BRCA1* mutant cell line, with end-point PCR confirming highly significant upregulation ($p < 0.0001$). hsa_circRNA_025636 showed stronger end-point PCR bands in both *BRCA1* and *BRCA2* mutants relative to wildtype, particularly in *BRCA2*, with qPCR confirming significant upregulation in *BRCA2* mutants ($p < 0.001$). These findings highlight a subset of circRNAs with differential expression across *BRCA* genotypes, potentially implicating them in *BRCA*-associated OC.

No.	circRNA	Group	End Point PCR Outcome	qPCR Outcome*	
				COV362 (BRCA1 mutant)	KURAMOCHI (BRCA2 mutant)
1	hsa_circRNA_400223	Common	Bands indicated upregulated in both <i>BRCA1</i> and <i>BRCA2</i> mutant compared to wildtype	*	**
2	hsa_circRNA_003300	Common	Bands appear in all three cell lines	**	Nos
3	hsa_circRNA_008026	Common	Bands appear stronger in both <i>BRCA1</i> and <i>BRCA2</i> mutant compared to wildtype, appearing strongest in <i>BRCA1</i>	**	*
4	hsa_circRNA_100059	Up in COV362 vs. OVSAHO	Bands appear darkest and most defined in COV362 (<i>BRCA1</i>)	****	Nos
5	hsa_circRNA_025636	Up in KURA vs. OVSAHO	Bands appear stronger in both <i>BRCA1</i> and <i>BRCA2</i> mutant compared to wildtype, appearing strongest in <i>BRCA2</i>	Nos	***

Table 6-8 CircRNAs for Further Validation After Primer Optimization and Validation

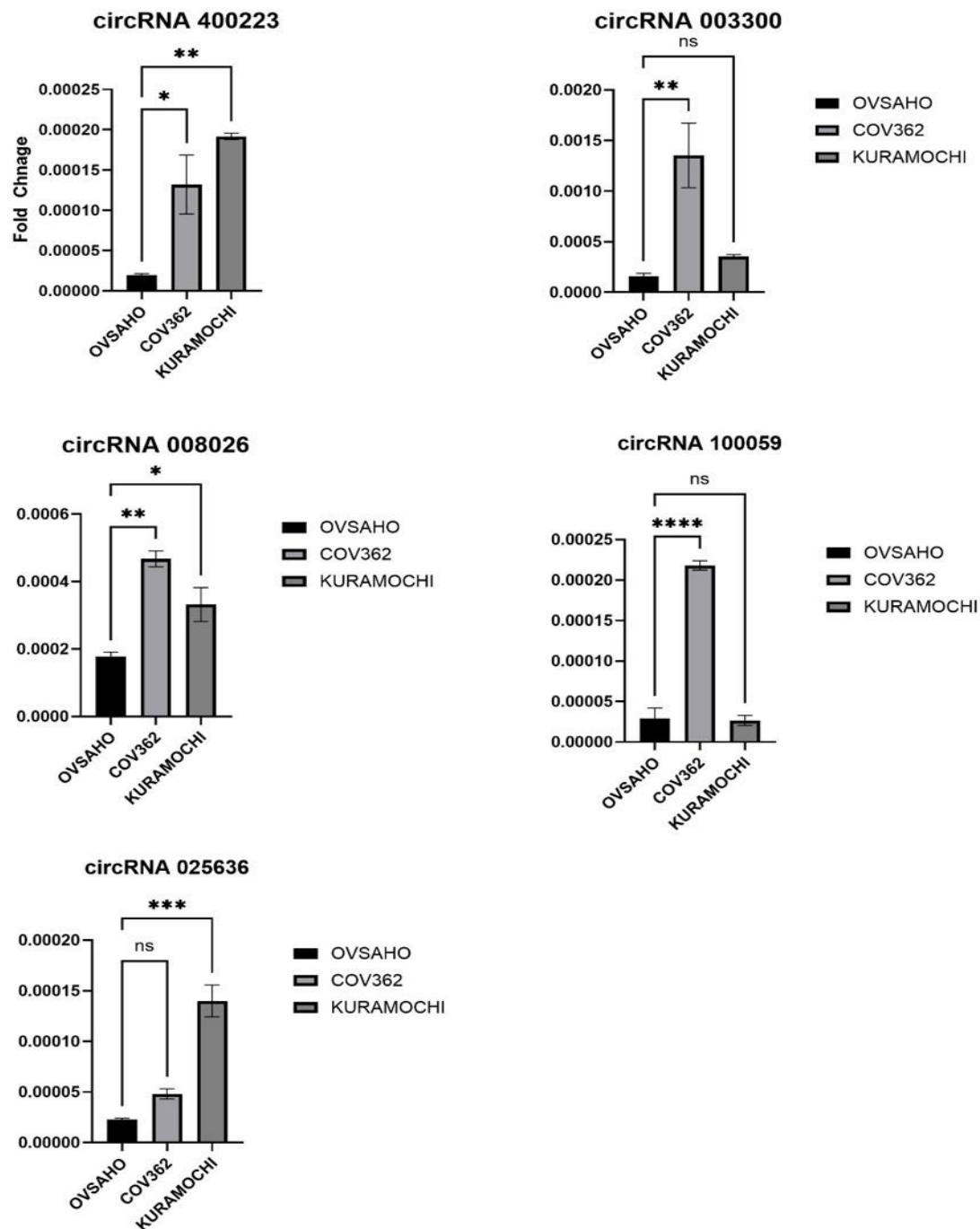


Figure 6-39 Validation of circRNAs in cell lines using qPCR

Expression of five circRNAs was quantified by RT-qPCR in OVSAHO (*BRCA*-wildtype), COV362 (*BRCA1*-mutant), and KURAMOCHI (*BRCA2*-mutant). circRNA expression was normalised to a housekeeping gene and calculated as fold change using the $2^{-\Delta\Delta C_t}$ method with OVSAHO as the calibrator. Bars represent mean $\pm$ SEM from technical triplicates ($n = 3$). Statistical comparisons across the three cell lines for each circRNA were performed using one-way ANOVA with Tukey's multiple-comparisons test; ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.000$

6.7.14 Evaluation of circRNA Patterns in a Panel of OC Cell Lines

Following initial validation in untreated *BRCA*-mutant and wildtype OC cell lines, the study next examined whether selected circRNAs were specifically associated with *BRCA* status or broadly expressed across an OC cell line model. Expression of five circRNAs (hsa_circ_400223, hsa_circ_003300, hsa_circ_008026, hsa_circ_100059, and hsa_circ_025636) was assessed across an extended panel including A2780 (wildtype), A2780cis (platinum-resistant), SKOV3 (chemoresistant, *BRCA* wildtype), and OVCAV8 (platinum-resistant, *BRCA* status unknown), as well as internally generated chemotherapy-resistant derivatives of COV362, KURAMOCHI, and OVSAHO (resistant to carboplatin/paclitaxel) (**Table 6-9**). Data from single replicate measurements ($n = 1$) demonstrated differential expression patterns (**Figure 6-40**). For example, hsa_circ_400223 and hsa_circ_008026 showed consistently elevated expression across *BRCA*-mutant and some resistant lines, suggesting possible relevance to both *BRCA*-associated and treatment-resistant OC phenotypes. In contrast, hsa_circ_100059 and hsa_circ_025636 appeared more selectively upregulated in *BRCA1*- or *BRCA2*-mutant models, respectively. No statistical comparisons were performed due to limited replicates, but these expression trends support further investigation of circRNAs as potential biomarkers of *BRCA* status or chemoresistance in OC. These were made resistant to the chemotherapy doublet Carboplatin and Paclitaxel, as described in the next chapter. This helped assess whether circRNA expression changed after resistance developed.

Cell Line	<i>BRCA</i> Status	Chemoresistance Profile
A2780	Wildtype	Platinum-sensitive
A2780cis	Wildtype	Platinum-resistant (cisplatin)
SKOV3	Wildtype	Chemoresistant
OVCAV8	Unknown	Platinum-resistant
COV362-Carbo/Taxol resistant	<i>BRCA1</i> mutant	Carboplatin and Paclitaxel-resistant (in-house)
KURAMOCHI-Carbo/Taxol resistant	<i>BRCA2</i> mutant	Carboplatin and Paclitaxel-resistant (in-house)
OVSAHO-Carbo/Taxol resistant	Wildtype	Carboplatin and Paclitaxel-resistant (in-house)

Table 6-9 OC Cell Line Panel and *BRCA* Status

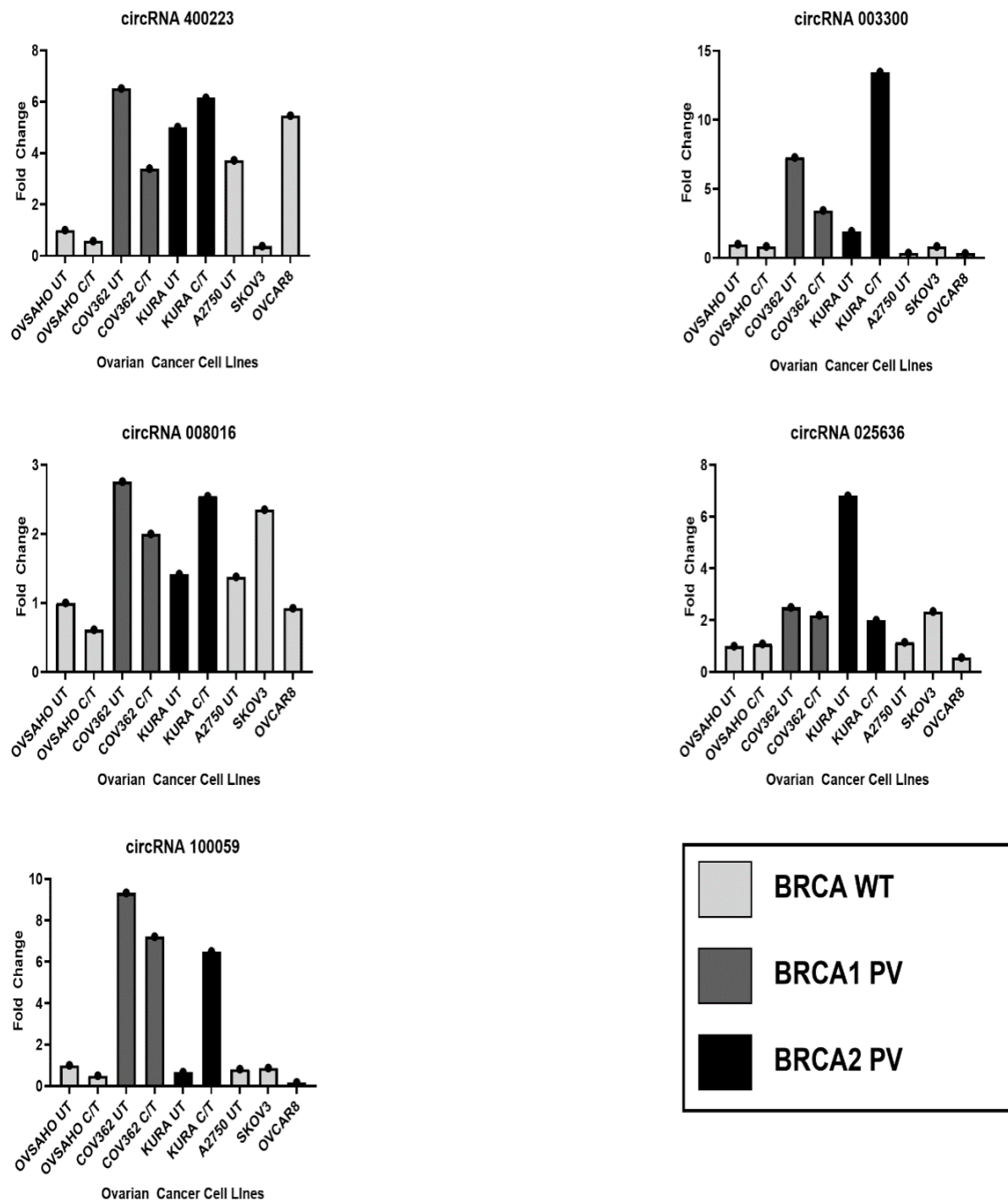


Figure 6-40 Fold-change expression of circRNAs across OC cell lines

Relative expression of five circRNAs was assessed by RT-qPCR across a panel of OC cell lines stratified by *BRCA* status: wildtype (WT), *BRCA1* PV, and *BRCA2* PV. Fold change was calculated using the $2^{-\Delta\Delta C_t}$ method with OVSAHO (*BRCA*-WT) as the reference. Bars represent mean $\pm$ SEM. Data represent single measurements per cell line ($n = 1$). No statistical comparison was performed.

6.7.15 Validation circRNA in clinical samples

To assess the expression of selected circRNAs in a clinical context, qPCR was performed on RNA derived from OC patient samples. This panel included cases harbouring a BRCA1 or BRCA2 CAV, with BRCA-wildtype samples used as controls. Comparisons between the wild-type control (TCDOG_1120) and individual BRCA1- or BRCA2-mutant samples were analysed using Dunnett's T3 multiple comparisons test. For circRNA_003300, circRNA_400223, circRNA_8026, and circRNA_100059, no statistically significant differences in expression were detected between the control and any BRCA-mutant sample (all adjusted $p > 0.05$; 95 % confidence intervals for all comparisons crossed zero).

For circRNA_025636, statistically significant differences were observed in six BRCA-mutant samples compared with the control (adjusted $p = 0.020$ – 0.049), with mean differences ranging from 0.66 to 0.99 and 95 % confidence intervals not crossing zero.

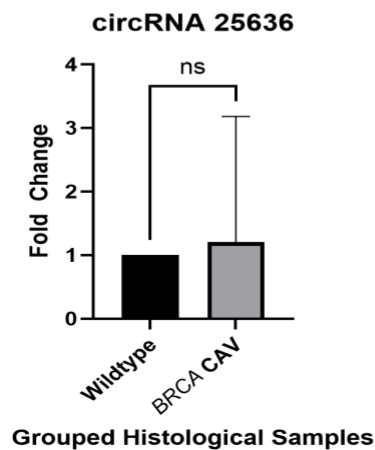
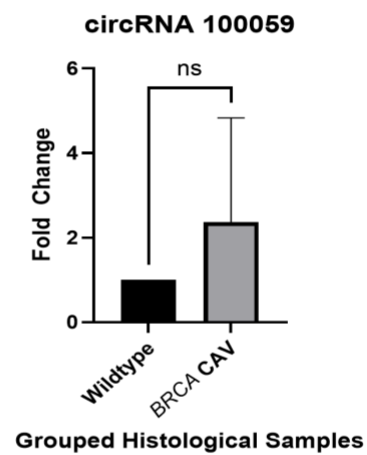
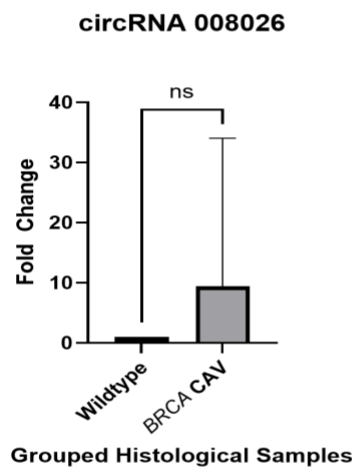
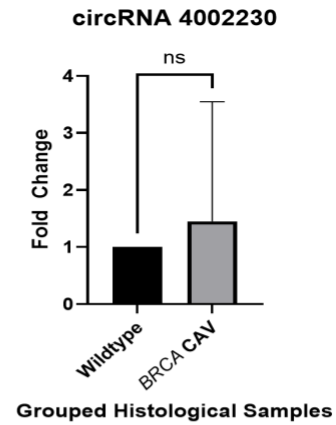
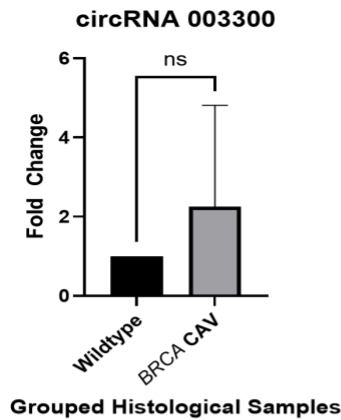


Figure 6-41 Validation of circRNA in Clinical Samples

Expression of selected circRNAs was assessed by qPCR in OC patient samples with *BRCA1* or *BRCA2* clinically actionable variants (CAVs) compared with *BRCA*-wildtype controls. Relative expression was compared using Dunnett's T3 multiple comparisons test, with bars representing fold change for individual samples relative to the wildtype control.

6.7.16 Exploring circRNA 025636

Expression of circRNA_025636 was validated in HGSOC models consisting of *BRCA1*, *BRCA2* and *BRCA*- wildtype cell lines. In the clinical validation cohort, circRNA_025636 was detected in 5 of 8 *BRCA* PV cases, with statistically significant lower detection in *BRCA*-wildtype comparators (**Error! Reference source not found.**). circRNA_025636 was identified as originating from the *RASSF8* locus gene (**Figure 6-42**), a gene implicated in cell adhesion and cytoskeletal organisation. In silico prediction revealed multiple high-confidence RNA-binding protein (RBP) interactions. Three distinct binding regions for *EIF4A3* were identified (positions 3–81, 93–196, and 137–264 bp), each showing 100% sequence identity with no mismatches or gaps. Additional predicted binding partners included AGO2 with two sites, and FMRP, FUS, HuR, and LIN28A with one site each.

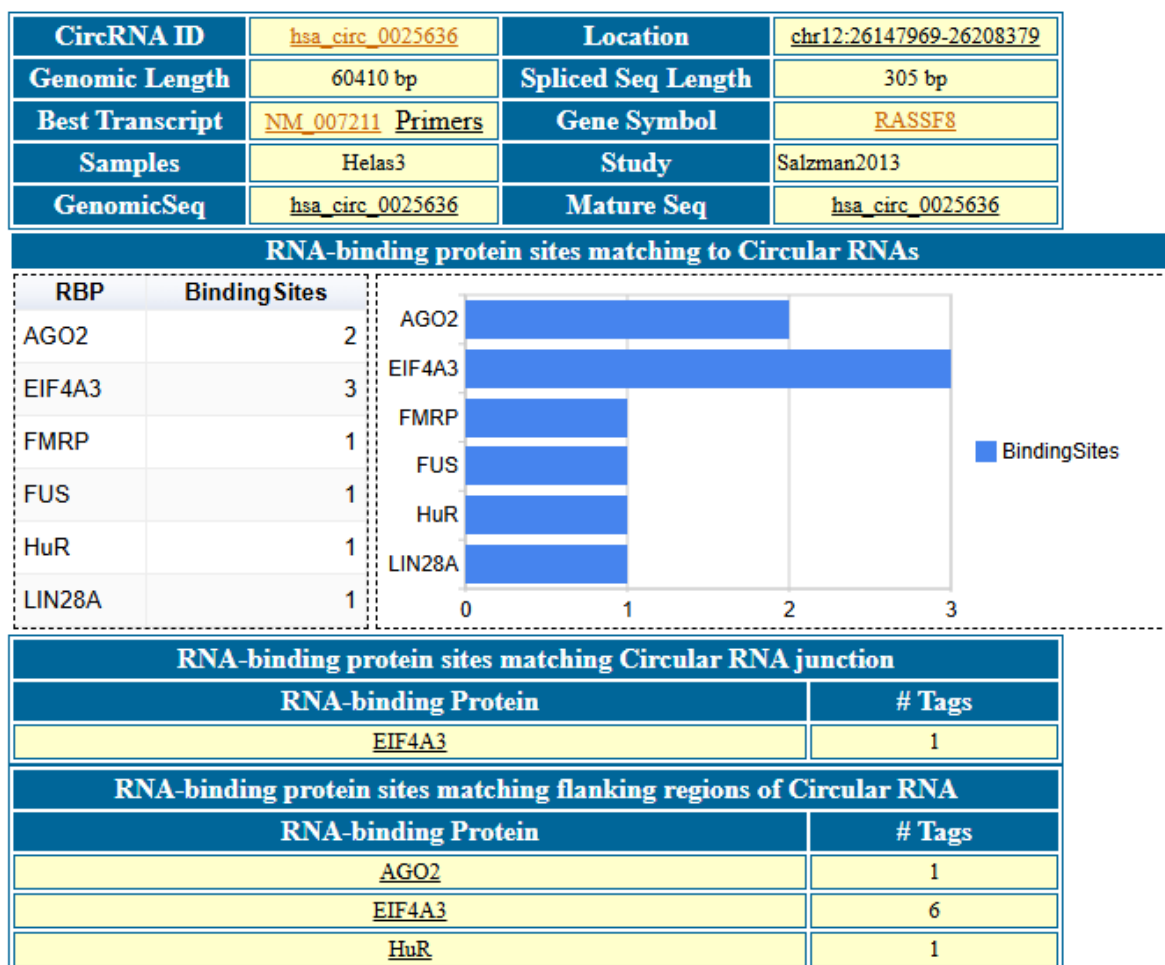


Figure 6-42 circInteractome summary for circRNA_025636 ([hsa_circ_0025636](#)).

Notably, FUS is a DNA-damage–response RBP that is rapidly recruited to DNA lesions in a PARP-dependent manner(431). Mutations in the FUS nuclear localization signal (NLS) impair Transportin-1–mediated nuclear import, depleting FUS from the nucleus and causing cytoplasmic accumulation, which in turn weakens PARP-dependent DNA-damage–response signalling and promotes aggregation (432). In parallel, FUS regulates circRNA biogenesis across cancers—e.g., circLONP2 (colorectal)(433), circZNF609 (lung)(434), and circHIF1A (TNBC)(435)—by binding intronic reverse-complementary sequences(431). Taken together, the predicted FUS–circRNA_0025636 interaction places this *RASSF8*-derived circRNA at a potential intersection between BRCA-associated DDR and adhesion/polarity pathways, motivating targeted validation of FUS binding and DDR-linked readouts in HGSOC.

STRING protein–protein interaction network for human *RASSF8* showing first-shell interactors enriched for cell polarity, tight junction and Hippo signalling (PARD3, PARD6A, PARD6B, PRKCI, TJP1, YAP1, FRMD6). Additional partners include TP53 pathway regulators TP53BP2 (ASPP2) and PPP1R13B (iASPP), as well as PPP1CA, PPP1CC, YWHAZ, and YWHAH. This placement indicates indirect connectivity to p53 signalling rather than a confirmed direct *RASSF8*–TP53 interaction. Edge thickness reflects interaction confidence and node colour indicates the query and first-shell proteins. Network generated with STRING v12.0. (**Figure 6-43**).

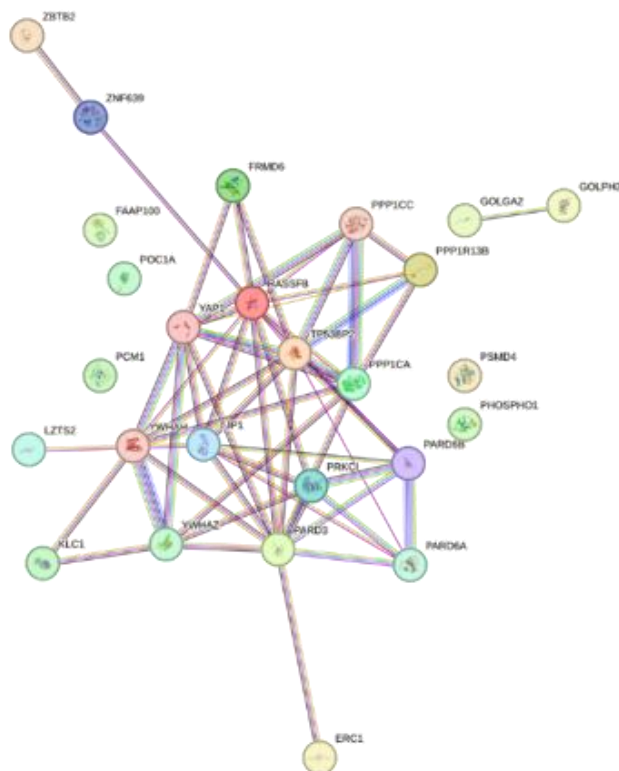


Figure 6-43 STRING network centred on *RASSF8* created using Genecards.org

6.8 Discussion

6.8.1 Cell Line Design Approach

The selection of the appropriate cell line is a critical step to ensure the integrity all subsequent experiments. Cell lines derived from tumours are the most frequently utilized models in cancer research and their use has advanced the understanding of cancer biology tremendously over the past decades (436). From the first culturing of HeLa cells in the 1950's, the scientific community has had a complicated journey with cell culture, and recently the utility of cell lines in translational and pre-clinical research has been questioned (437, 438). The seemingly straightforward step of cell line selection, becomes increasingly complex given the divergent information available in the literature (439). Even if researchers start out with a cell line they believe to be representative, they may not be representing the cancer of origin nor the cancer subtype (i.e. grade) (437, 440). Technological advances has facilitated the molecular profiles of hundreds of cell lines from The Cancer Cell Line Encyclopaedia and thousands of tumour samples from the Cancer Genome Atlas, studies have revealed that popular cell line models often do not accurately represent their corresponding tumour types (184). Even if the appropriate cell line is selected, cross-contamination and microbial contamination are also a problem (269), therefore a cell authentication is now recommended. While a variety of methods exist for authentication testing, short tandem repeat (STR) profiling has been endorsed as the most widely used method currently available for human cell lines (441).

Issues with cell line selection has been reported as a particular problem in OC cell lines. Disturbingly, studies have revealed that the most frequently used cell lines are largely poorly suited for investigating HGSOC (184). Conversely, cell lines that more closely resemble the most common tumour types (HGSOC) are rarely used in laboratories (46, 184). Importantly, studies by Domcke et al. and McCabe et al. demonstrate that the limited applicability of preclinical models is further compounded when histological subtype is overlooked, underscoring the need to select appropriate cell lines to maximize successful translation to the clinic. This has not been without huge cost of resources and energy and poor model selection may have contributed to several failed clinical trials (184, 442). This has highlighted the need for subtype-specific research in OC and fastidious selection of appropriate cell lines (46).

This further calls into question the scientific robustness of previous OC circRNA studies conducted using poorly selected cell lines as models of HGSOC. **Table 6-10** demonstrates that the most frequently used cell lines in circRNA studies have been ranked poorly as models for HGSOC, indicating that the findings may be compromised due to their limited representativeness of the actual disease. This discrepancy highlights the need for more accurate and clinically relevant models to ensure that circRNA-based insights are truly reflective of HGSOC pathology and can be effectively

translated into therapeutic strategies. Further, the most frequently profiled drug relating to circRNA is cisplatin (93, 127, 128, 130, 135, 136, 137), while there is a dearth of data on circRNA in carboplatin treated OC. As outlined above, carboplatin is favoured in many regions due to its favourable toxicity profile. Further there no data relating to circRNA as a biomarker of *BRCA* in OC.

Cell Line Used in circRNA studies	Frequency of use in assessing biomarkers	Frequency of Use in assessing resistant models	Ranking out of 47 of likely HGSOCa as per Domcke Paper (184)	Assessment of HGSOCa as per McCabe Paper (46)
A2780	6	3	42	Not recommended for use
SKOV3	8	4	41	Clear Cell OC model
HO8910	2	0	Not ranked	Not ranked
CAOV3	2	1	Not ranked	HGSOC Model
OVCAR-3	3	2	13	HGSOC Model
OV1063	1	0	Not ranked	Not ranked
TOV21G	0	1	47	Clear Cell OC model
COC1	0	1	Not ranked	Not ranked

Table 6-10 Cell lines used in circRNA in OC literature above

Therefore, the selection of COV362, KURAMOCHI and OVSAHO in this study was deliberate, as these models have been shown to more closely recapitulate the genomic and transcriptional features of HGSOC compared to commonly used but poorly ranked lines such as A2780 and SKOV3 (443). In fact, it has been explicitly reported that KURAMOCHI and OVSAHO provide more accurate models of HGSOC than more commonly used SKOV3 (444). Notably, nearly 60% of ovarian carcinoma studies have utilised only A2780 and SKOV3, with approximately 90% of published work relying on just five SOC cell lines (445), underscoring the need for broader representation. The use of three well-characterised HGSOC models encompassing *BRCA*-wildtype and *BRCA1* PVs backgrounds provides a robust foundation for this study.

6.8.2 Expression Patterns of *BRCA*-Linked circRNAs

The hierarchical clustering heatmaps, scatter plots and volcano plots collectively provide insight into the complexity of circRNA regulation across *BRCA1*-mutant, *BRCA2*-mutant and *BRCA*-wildtype

HGSOC models. The absence of consistent clustering by *BRCA* genotype in the global heatmap suggests that circRNA expression is not dominantly shaped by *BRCA* status. Instead, cell line specific differences were more apparent, indicating that circRNA expression patterns may be influenced by a multitude of factors rather than a single mutational driver. Within such a complex biological system, it is unlikely that circRNA expression patterns would segregate exclusively by *BRCA* status.

Pairwise comparisons refined these observations. Distinct clustering by cell line in COV362 vs. OVSAHO and KURAMOCHI vs. OVSAHO comparisons pointed to model-specific effects rather than shared *BRCA*-driven profiles. Even in the direct *BRCA1* vs. *BRCA2* comparison, expression remained heterogeneous. Such findings suggest that circRNAs may act downstream of multiple converging regulatory mechanisms, and their expression may be modulated by transcriptional, epigenetic or microenvironmental cues in addition to mutational status.

Scatter plots and volcano plots helped to contextualise these global findings by identifying discrete subsets of circRNAs with differential expression between specific models. While most circRNAs were evenly expressed across cell lines, a smaller fraction showed marked up- or downregulation, consistent with the presence of circRNA subgroups that could function as biomarkers or effectors in *BRCA*-related biology. Importantly, the identification of such candidates supports the rationale for focused validation in independent cell lines or clinical cohorts, where biologically relevant patterns may emerge more clearly against reduced background noise.

Taken together, these results illustrate that circRNA profiling in HGSOC reveals both complexity and selectivity: global clustering patterns are obscured by biological and technical variability, but subsets of circRNAs with potential relevance to *BRCA* biology can still be discerned. These findings mirror the clinical heterogeneity observed in *BRCA*-mutant OC where penetrance, modifying alleles and therapy responses vary considerably between patients. Interpretation may also be confounded by in vitro factors such as passage number, culture conditions and clonal selection. Validation in larger cell line panels and independent clinical cohorts will be essential to clarify whether specific circRNAs consistently associate with *BRCA* status. That said, the absence of global clustering by *BRCA* status does not preclude the existence of individual circRNAs that may serve as representative markers of *BRCA*. Targeted validation of such candidates will be important to determine whether specific circRNAs can reliably reflect *BRCA*-associated biology despite broader background variability.

6.8.3 Functional Landscapes of *BRCA*-Linked circRNAs

Analysis of upregulated circRNAs across *BRCA1*-mutant (COV362), *BRCA2*-mutant (KURAMOCHI), and *BRCA*-wildtype (OVSAHO) cell lines revealed distinct patterns of functional enrichment across BPs, cellular components, and molecular functions. CircRNAs upregulated in *BRCA1* mutant cells were primarily associated with signalling regulation, developmental pathways and exosome-related

components, suggesting roles in tumour signalling dynamics and intercellular communication. In contrast, circRNAs upregulated in *BRCA2*-mutant cells were enriched in processes related to cytoskeletal organisation, focal adhesion and intracellular binding activity, indicating potential involvement in structural remodelling and spatial regulation. Direct comparison between *BRCA1* and *BRCA2* models highlighted further divergence, with *BRCA1*-associated circRNAs linked to chromosome segregation, morphogenesis and kinase signalling, while *BRCA2*-linked profiles suggested a more adhesion and organisation focused expression pattern. Collectively, the data indicate that circRNA programmes in HGSOC track with *BRCA* context rather than forming a single shared signature. *BRCA1* models preferentially enrich signalling, cell-cycle/segregation and exosome terms, whereas *BRCA2* models skew toward cytoskeleton, focal adhesion and intracellular binding, consistent with differences in DNA-repair vs. adhesion/architecture biology. The chromosomal distribution likewise argues for broad genomic influences on circRNA biogenesis: recurrent signals on chromosomes 1, 2 and 17, with chr17 enrichment in *BRCA1*-mutant cells (*BRCA1* at 17q21) suggesting local (cis) effects, while the absence of clustering on chr13 in *BRCA2* models points to trans or distinct regulatory mechanisms. These patterns align with the concept that circRNA formation is shaped by regional transcriptional activity and chromatin state rather than isolated mutational hotspots.

These observations support the view that circRNAs encode pathway-level states that refine genotype-based stratification and may expose differential vulnerabilities (e.g., homologous-recombination-linked vs. adhesion/FAK-centred dependencies). They also help reconcile clinical heterogeneity within *BRCA*-mutant disease, where similar phenotypes can result from different underlying regulatory mechanisms. Given the hypothesis-generating nature of enrichment analyses, causality should be tested using targeted knockdown/overexpression of representative circRNAs with pathway-matched readouts, and effects should be cross-validated across additional HGSOC models and independent cohorts. Integration with transcriptomic and proteomic datasets will be important to define downstream networks and to prioritise candidates with translational potential, including those that may inform PARP-inhibitor response or adhesion-directed therapeutic strategies.

6.8.4 Biological Interpretation of BRCA-Linked KEGG Pathways

KEGG pathway analysis across *BRCA*-stratified models revealed enrichment in a range of signalling and proliferative pathways. In *BRCA1*-mutant COV362 cells, circRNAs mapped to pathways involved in cytoskeletal regulation, ErbB signalling, and glioma. While glioma is a central nervous system malignancy, the enrichment likely reflects shared oncogenic signalling components such as *EGFR* and *PIK3CA* that are relevant in OC. These findings suggest that *BRCA1*-associated circRNA upregulation may influence tumour cell communication, survival and progression.

In *BRCA2*-mutant KURAMOCHI cells, the enriched pathways highlighted a distinct pattern involving focal adhesion, proteoglycans in cancer, and membrane signalling. This suggests that circRNAs upregulated in *BRCA2*-mutant cells may contribute to cellular adhesion, structural organisation, and stress signalling, indicating a divergent circRNA landscape compared to *BRCA1*-mutant OC. When comparing COV362 to KURAMOCHI directly, enrichment of DNA replication, cell cycle and mismatch repair pathways was observed in *BRCA1*-mutant cells. These findings align with the known role of *BRCA1* in maintaining genomic stability(446) and suggest that *BRCA1*-linked circRNAs may participate in transcriptional regulation, proliferative signalling and DNA damage response. The pathways identified are directly relevant to established hallmarks of OC, including proliferative signalling, genomic instability and altered cell adhesion. These results suggest that circRNA profiles could eventually inform therapeutic stratification, for example identifying patients more likely to benefit from PARPi or adhesion-targeted therapies. Nonetheless, KEGG analyses should be interpreted cautiously given pathway redundancy and the risk of false discovery, reinforcing the need for cross-validation in larger datasets.

6.8.5 circRNAs as Biomarker of *BRCA*

The search for molecular biomarkers of genetic alterations is well established, with extensive efforts focused on nucleic acids and proteins(447). Among these, miRNAs have been the most extensively investigated, with several examples reported across different tumour types. In colorectal cancer (CRC), miR-135a/b are upregulated and target tumour suppressor gene Adenomatous Polyposis Coli (APC), promoting Wnt activation(448). miR-21 is widely overexpressed and prognostic in CRC and has been associated with oncogenic signaling contexts (e.g., BRAF/KRAS pathways), though its relationship to specific TP53 or KRAS mutation status varies by cohort (141, 449). To date, no miRNA-based cancer panel has FDA approval, although several laboratory developed tests / assays with FDA Breakthrough Device designation are in evaluation(450). Specifically for *BRCA*, a number of miRNA have been put forward as potential biomarkers, however these require extensive validation (402). For example, miR-182 has been shown to be upregulated in *BRCA1* compared to wild-type in breast cancer (404, 412). While much attention has been given to miRNAs as biomarkers over the past two decades, circRNAs are only beginning to be investigated extensively, and virtually no literature exists on circRNAs as biomarkers of *BRCA* status. Indeed, a focused review of the published literature reveals no reports describing circRNAs as biomarkers in the context of *BRCA* mutations, underscoring the novelty of this work. Nevertheless, circRNAs have become the focus of an expanding body of research where their biological roles and potential clinical applications are increasingly being recognised. For example, circPDIA3 has been shown to be upregulated in CRC, associated with oxaliplatin resistance and worse survival, and reduces cell death by blocking GSDME-driven

pyroptosis, and works in a miR-449a/XBP1 loop (451). Therefore, circPDIA3 may represent one of the first novel circRNA targets to overcome chemoresistance in CRC patients. Beyond oncology, circRNAs have emerged as predictive biomarkers for drug response in psychiatry, notably, a brain-enriched blood circRNA has been reported to predict SSRI outcomes (e.g., sertraline) in the EMBARC cohort, with an LDT now offered clinically, underscoring translational potential for treatment selection in depression(452).

Despite the growing volume of publications, the translation of circRNA research from bench to bedside remains challenging due to several common pitfalls. Beyond the inherent limitations of cell line models and challenges in clinical translation previously discussed, additional technical and methodological hurdles exist specific to circRNA. Key issues include the lack of standardised methodologies for circRNA detection and validation, the risk of false positives arising from rolling-circle amplification or template switching during reverse transcription, and insufficient use of rigorous negative controls such as RNase R treatment to confirm circularity(453). Additional barriers include the variability introduced by differences in library preparation, bioinformatic pipelines and reference genome annotations, as well as the frequent absence of validation across independent cohorts or clinical specimens. Furthermore, studies often underreport essential methodological details, hindering reproducibility and comparability between datasets. In this work, several strategies were implemented to mitigate these risks. Sequencing was outsourced to Arraystar, a specialist provider with established circRNA profiling pipelines and quality control processes, ensuring consistency in data generation. Candidate circRNAs were prioritised based on robust sequencing metrics, and validation was performed using specifically designed divergent primers. Despite these measures, challenges inherent to working with circRNAs in clinical samples, such as RNA degradation and variability in sample quality, remain relevant. Nevertheless, recent reports have demonstrated the successful application of circRNAs in clinical investigations(451), underscoring their potential value in diagnosis, prognosis, and treatment stratification. In this study, qPCR analysis of selected circRNAs in clinical OC samples identified statistically significant differences only for circRNA_025636, which showed elevated expression in a subset of *BRCA*-mutant cases. For the other circRNAs tested, no significant differences were detected. Interpretation of these findings is limited using a single biological replicate per patient sample, which reduces statistical power and the ability to confirm reproducible differences in expression. Additionally, RNA extracted from archival patient samples can be of variable quality and integrity, particularly when derived from formalin-fixed paraffin-embedded tissue, potentially affecting detection sensitivity. These factors, together with the inherent instability of RNA in clinical material, may contribute to variability and hinder the identification of statistically robust differences in expression.

6.8.6 circRNAs 0025636

Prior studies indicate that RASSF8 restrains OC progression via miRNA pathways, with circCERS6 relieving miR-630-mediated repression of RASSF8 (454) and miR-320a promoting proliferation and invasion by directly targeting RASSF8 (455). In this context, the identification of the RASSF8-derived circRNA_025636, its predicted AGO2 and EIF4A3 interactions, and its detection in a subset of *BRCA* PV cases suggest a circRNA-based extension of the RASSF8 axis that merits biomarker and mechanistic evaluation in HGSOC. Notably, EIF4A3 has been reported to regulate circRNA biogenesis in several cancers, including circSEPT9 in breast cancer(456, 457), circMMP9 in glioblastoma(458), and circ_0084615 in colorectal cancer(459), suggesting that its interaction with circRNA_025636 may have functional relevance. Taken together, these findings suggest that circRNA_025636 may participate in post-transcriptional regulation via EIF4A3 and other RBPs, with potential downstream roles in adhesion, migration, and proliferative signalling in HGSOC. The positioning of RASSF8 alongside ASPP family regulators of p53 (**Figure 6-43**), together with the near universal alteration of TP53 in HGSOC, is consistent with functional crosstalk between adhesion and polarity pathways and stress responses mediated by p53. This linkage provides a plausible route by which the RASSF8-derived circRNA_025636 could intersect with invasion, epithelial integrity, and DNA damage programmes in OC.

6.8.7 Conclusion

This study profiles circRNA expression across *BRCA* mutant and wild-type HGSOC cell lines with subsequent validation in models and clinical samples. Strengths include use of divergent primers, independent cross-model validation and integration of experimental and patient-derived material. Limitations include a small clinical cohort, single biological replicates and variable RNA quality which constrain definitive biomarker inference. Nonetheless, detection of circRNA_025636 in a majority of *BRCA* mutant cases supports the potential of circRNAs as biomarkers; prospective validation in larger cohorts, multi-omic integration and targeted functional studies are warranted to establish mechanism and clinical utility.

More broadly, the work underscores the importance of rigorous model selection, as poorly ranked or histologically inappropriate lines can mislead interpretation. While global clustering by *BRCA* genotype was not observed - highlighting the complexity of circRNA regulation- discrete circRNA subsets with biomarker potential were identified, and functional/pathway analyses revealed distinct *BRCA1* vs. *BRCA2* linked regulatory landscapes. Collectively, the findings suggest circRNAs can enhance biological understanding and stratification in *BRCA*-mutant HGSOC, while emphasising the need for careful model choice, robust validation and clinical translation.

The Development of Resistant OC Models: Lessons and Limitations

Chapter Seven

7 The Development of Resistant OC Models: Lessons and Limitations

7.1 Introduction

The development of treatment resistance allows for the proliferation of cancer cells, disease progression, and ultimately, death (228). Tumours which develop resistance to drug therapies demonstrate either innate or acquired resistance. Understanding the molecular basis of this resistance is crucial for rationalizing drug selection strategies in specific patient populations, thereby improving outcomes (460). By studying these resistance models, scientists can gain insights into the biology of mechanisms of resistance to these drugs (461). This facilitates advancements in testing new drugs and/or drug combinations and exploring strategies to overcome resistance. A drug-resistant cell line refers to a clone of cells with characteristic pathology that have developed resistance to the effects of the pharmacological agents typically used to treat these types of cells (462).

The creation of drug-resistant cancer cell lines is a long-recognized methodology for examining the *in vitro* mechanisms of resistance to chemotherapy agents (270, 437). When developing a resistant cell line model researchers must make key strategic decisions regarding methodology. Drug-resistant cell models are artificially created in the laboratory by exposing cells to chemotherapeutic agents using either gradually increasing concentrations over time or a single high-dose pulse. The drug sensitivity of these surviving cells is then compared to age matched parental cells. In high-level laboratory models, drug doses are escalated without a defined upper limit, provided that some cells survive to establish resistance. Conversely, clinically relevant models require a thorough understanding of clinical treatment protocols. For these models, pharmacokinetic studies are essential to determine the drug concentration levels achieved in the bloodstream.

Beyond the challenge of late-stage detection, one of the leading contributors to poor prognosis in OC is the development of resistance to platinum agents (460). Clinically, chemotherapy-resistant OC arises due to the overexpression of certain proteins and modifications in signalling pathways PI3K/AKT/mTOR, MAPK/ERK, and NF- κ B, which promote cell survival, inhibit apoptosis, and enhance DNA repair capacity (228, 463). Dysregulated genes associated with drug resistance can influence cellular mechanisms including drug efflux, apoptosis, and the repair of DNA damage (228). Additionally, cancer stem cells (CSCs) within OC significantly contribute to the development of chemoresistance (460).

However, challenges remain in ensuring that *in vitro* resistance models accurately reflect the complexity of the tumour microenvironment, architecture and heterogeneity (464). For example,

immune cells, stromal cells, and blood vessels are absent in traditional 2D cell culture. Furthermore, there is concern that cell lines may change their characteristics and genetic features after prolonged culture (270). Three-dimensional (3D) culture systems, including spheroids and organoids, have been developed to better recapitulate tumour architecture and cell–cell interactions, offering a more physiologically relevant alternative to 2D models (465). Nevertheless, cell models have long been universally adopted as a springboard for translational studies due to their accessibility, relative ease of management and affordability. Furthermore, scientists can be highly selective in choosing cell lines that represent a particular disease state (436). The literature reports that developing a drug-resistant cell line can take up to 18 months, yet studies often place little emphasis on the methodological background or selection strategy used to establish these cell lines (270). Given these factors, creating a resistant cell line model can be a challenging process that necessitates careful planning and consideration. One of the main challenges lies in reproducing clinically relevant drug exposure, as the concentrations and treatment durations used in vitro often exceed those achievable in patients, limiting the physiological relevance of resistance mechanisms observed (270, 466). To address the persistent clinical challenge of treatment resistance in OC, this chapter details the generation and characterization of resistant cell line models across three representative OC cell lines. These models were developed to reflect resistance emerging in patients treated with chemotherapy and PARPi, providing a translational link between clinical observations and laboratory investigation.

7.2 Aims and Objectives

7.2.1 General Aims

This chapter focuses on the development of chemotherapy and PARPi resistant OC cell lines.

7.2.2 Hypothesis

The hypothesis is that resistance to chemotherapy (single-agent and doublet) and PARPi can be independently induced in OC cell lines, and that these models will exhibit distinct molecular and phenotypic profiles.

7.2.3 Objectives

The objective of this chapter is to:

- Establish and validate chemotherapy- and PARPi-resistant OC cell line models
- Document methodological differences, timelines, and limitations across treatment classes
- Characterise biological features and adaptive mechanisms associated with resistance

7.3 Results

7.3.1 Strategies for Generating Resistance Models

7.3.1.1 Selection of resistant model

Typically, there are two primary avenues to approach drug exposure - a clinically relevant model or a high-level laboratory model (270), characteristics of which are outlined in Table 7-1.

High Level Laboratory Model	Clinically Relevant Model
• Doses of drug are often high, and doses are escalated over time • Cells are frequently cultured continually in the presence of drug or highly drug-resistant clones are selected from a mixed population • High-level models are often more stably resistant and therefore easier to maintain in culture for ongoing research • Levels of resistance are often higher and as such molecular changes associated with the mechanism of resistance are more pronounced	• Tries to imitate the clinical setting as much as possible • Cells are exposed to 'pulses' of drug exposure, mimicking how patients receive treatments • This can select for cells that survive and proliferate despite repeated drug challenge • Disadvantages to clinically relevant models can include unstable resistance, very low-level resistance, and less discernible molecular changes • Drug resistance can be defined as a five-fold increase in the IC₅₀ value of resistant cell line compared to the parent cell line

Table 7-1 Clinical vs. laboratory resistance models: Key Features

While these models are a good blueprint to springboard efforts to develop a resistant model, it has been acknowledged that the reality is that most selection strategies begin as a clinically relevant model and then evolve into a high-level model due to escalating drug doses (270). This is primarily because of the stability of the resistance phenotype produced by a high-level laboratory model.

7.3.1.2 Selection of therapeutics

Therapeutics were selected to closely replicate the clinical setting. The standard systemic treatment for advanced HGSOc involves an intravenous infusion of a Carboplatin and Paclitaxel chemotherapy doublet, making these drugs an appropriate choice for the experimental model. In clinical practice, this regimen is administered every three weeks for six cycles. According to NCCP guidelines, the dosages used are Carboplatin (AUC5-7.5) and Paclitaxel 175 mg/m² (271) (**Figure 7-1**). Typically, a Carboplatin AUC6 is used in clinical settings, with the dose calculated using the formula: Dose (mg) = target AUC (mg/mL x min) × (GFR mL/min+25).

Admin. Order	Day	Drug	Dose	Route	Diluent & Rate	Cycle
1	1	PAClitaxel	175mg/m ²	IV infusion	500ml 0.9% NaCl over 3 hours	Every 21 days for 6 cycles
2	1	CARBOplatin	AUC (5-7.5)	IV infusion	500ml glucose 5% over 30 min	Every 21 days for 6 cycles
PAClitaxel must be supplied in non-PVC containers and administered using non-PVC giving sets and through an in-line 0.22 µm filter with a microporous membrane.						
PAClitaxel should be diluted to a concentration of 0.3-1.2mg/ml.						

Figure 7-1 NCCP Chemotherapy Regimen for Carboplatin / Paclitaxel

Taken from source (467)

The selection of PARPi was less straightforward than chemotherapy. Section 1.1.7.3 outlined the various clinical trials supporting the use of several PARPi's in HGSOc, namely olaparib, niraparib, rucaparib and veliparib. The licensing for these drugs varies internationally, specifically for niraparib, which has recently has its FDA approval revoked for all comers (*BRCA* wildtype), although it has maintained its European Medicines Agency (EMA) approval (77). A recent meta-analysis reported no statistically significant difference in efficacy among olaparib, niraparib and rucaparib, though the study highlighted that olaparib may cause fewer serious adverse events (468). Therefore, olaparib is the treatment of choice in the Irish setting, and the PARPi selected for follow up experiments.

7.3.1.3 Selection of dose of administration

The choice of treatment doses depends on whether a clinically relevant model or a high-level laboratory model is being used. To establish an accurate clinically relevant dosing strategy for *in vitro* models, the following steps should be followed (270):

Step One: Determine Human Dose

- Refer to the National Cancer Control Program (NCCP) national Systemic Anti-Cancer Therapy (SACT) guidelines for publicly available doses in Ireland
- Identify the human dose for intravenous (I.V.) administration in mg/m²

Step Two: Account for Pharmacokinetics

- Conduct a literature review to gather information on drug levels in the bloodstream from typical dosing over time

Step Three: Determine Treatment Dose

- Calculate molarity from mass (peak plasma level), formula weight (molecular weight), and volume using online molarity tools like GraphPad (469).

Step Four: Validate Doses Through IC50 Calculations

- Validate doses empirically by calculating IC50 values through cell viability and fitting dose–response curves.
- Ensure doses span a range between IC20 – IC80.

Step Five: Monitor Response

- Acknowledge that biological models may not replicate human models exactly due to pharmacokinetics, metabolism, and drug distribution complexities.
- Treat models and carefully monitor responses and interpret results considering these limitations.

Step Six: Adapt Dose as Needed

- After administering the dose to the *in vitro* model, monitor its effects.
- Adjust the dosing regimen based on observed responses and toxicity.
- If no cells survive, lower the dose accordingly.

7.3.1.4 Dose Optimisation for High Level Laboratory Model

To establish an appropriate starting point for drug exposure in the high-level laboratory resistance model, dosing was initially informed by clinical pharmacokinetic data, as outlined above and calculated in **Appendix 7**. This approach allowed for a physiologically relevant baseline from which escalating drug concentrations could be trialled. A target optimum dose was calculated for each therapeutic agent using peak plasma concentrations and pharmacokinetic data from clinical settings (Error! Reference source not found.). Following administration, plasma concentrations of these agents decline rapidly, reflecting their short systemic exposure. After a 375 mg/m² intravenous bolus of carboplatin, peak plasma levels reach approximately 39 µg/mL before falling to 9 µg/mL within 2 hours (272). Paclitaxel achieves peak plasma concentrations of up to 10,000 ng/mL, decreasing to 50 ng/mL within 24 hours (274). Similarly, after a single 400 mg dose of olaparib, plasma concentrations peak at around 7 µg/mL and fall below 0.1 µg/mL after 50 hours (278). These doses, summarised in **Table 7-2**, were used to guide the initial exposure concentrations.

Treatment	Peak Plasma Concentration	Target Optimum Dose
Carboplatin	39 µg/mL	24.3 (µM)
Paclitaxel	10,000 ng/mL,	58.6 (nM)
Olaparib	7 µg/mL	16.1 (µM)
Carboplatin / Paclitaxel	39 µg/mL / 10,000 ng/mL	24.3 (µM) / 58.6 (nM)

Table 7-2 Dose Optimisation for Treatment Summary

9.1.1 Preliminary Data Guiding Development of Clinically Relevant Resistance Models

Target doses were determined using peak plasma concentrations and pharmacokinetic data from clinical settings as previously described. Cells were exposed to the respective agents for 24–72 hr and subsequently monitored for up to 10 days post-treatment to assess cytotoxicity and recovery.

7.3.1.5 Drug Sensitivity and Recovery in COV362

Qualitatively, COV362 demonstrated a differential response across drug types and concentrations. Cytotoxicity and recovery were assessed qualitatively through daily microscopic observation of cell morphology and confluence. Evidence of recovery was defined by the reappearance of adherent, proliferating cells following initial cytotoxic effects. The lowest carboplatin dose (6 µM) produced minimal cytotoxicity, with cells regaining confluence within five days, suggesting subtherapeutic exposure. The intermediate dose (12 µM) induced partial cytotoxicity, characterised by reduced confluence and delayed recovery over approximately eight days, indicating a more effective yet tolerable exposure. The highest dose (24 µM) caused marked cell death (**Figure 7-2**). In contrast, all doses of paclitaxel (15 nM, 30 nM, 60 nM) were excessively toxic in COV362, causing near-complete cell death with no significant recovery observed during the 10-day follow-up. The most promising condition was the lowest dose of olaparib (16 µM), which triggered an initial cytotoxic response followed by notable cellular recovery beginning around day 7. Based on this response profile, this condition was selected for a repeat treatment at day 10 post-initial exposure to explore effects of re-challenge.

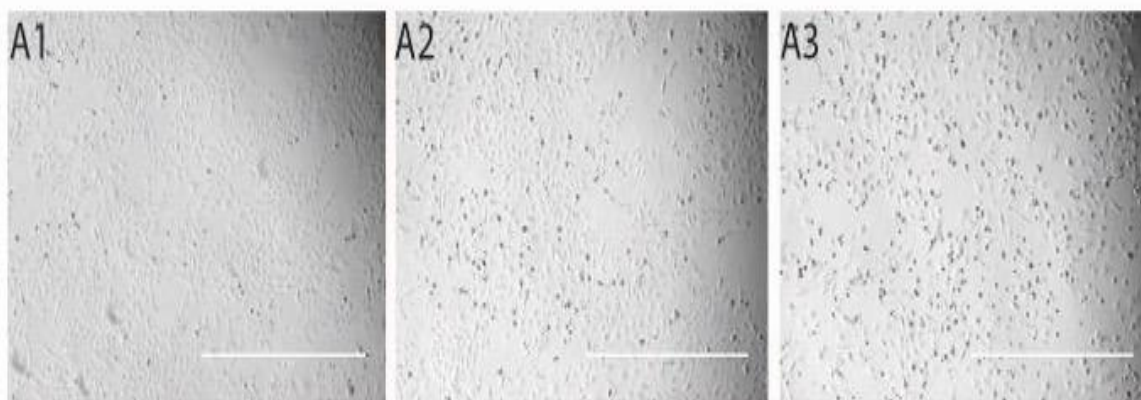


Figure 7-2 COV362 cells showing increased cell death with escalating carboplatin concentrations

A1 = 6 μ M carboplatin, A2 = 12 μ M carboplatin, A3 = 24 μ M carboplatin. Cells exhibited dose-dependent cytotoxicity with progressive loss of confluence. Images captured under phase-contrast microscopy at $\times 10$ magnification.

7.3.1.6 Drug Sensitivity and Recovery in KURAMOCHI

KURAMOCHI cells demonstrated hypersensitivity to all treatment conditions as judged by pronounced cytotoxicity, loss of confluence, and absence of recovery during the post-treatment period. Even the lowest doses of single-agent carboplatin, paclitaxel and olaparib caused widespread cell death within 72 hr, with no appreciable recovery over the 10-day post-treatment window. No surviving population was robust enough to consider re-treatment. The experiment was subsequently repeated using lower carboplatin doses (2 μ M, 4 μ M, and 6 μ M) to identify a sublethal exposure range (**Figure 7-3**).

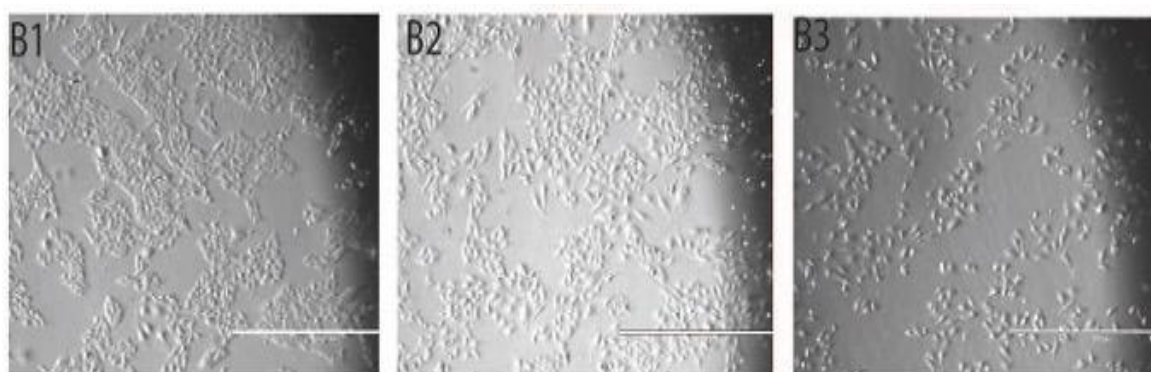


Figure 7-3 Kuramochi cells showing response to escalating carboplatin concentrations.

A1 = 2 μ M carboplatin, A2 = 4 μ M carboplatin, A3 = 6 μ M carboplatin. Cells exhibited marked cytotoxicity with minimal recovery, as judged by loss of confluence. Images captured under phase-contrast microscopy at $\times 10$ magnification.

7.3.1.7 Drug Sensitivity and Recovery in OVSAHO

OVSAHO cells exhibited a favourable response to the lowest dose of carboplatin (6 μ M), with partial cell death observed at 72 hr and complete morphological recovery by day 9. These cells were subsequently rechallenged with the same dose to assess cumulative response. Higher doses of carboplatin (12 μ M and 24 μ M) resulted in irreversible cytotoxicity, with negligible recovery observed after 14 day post-treatment period. All concentrations of paclitaxel tested (15–60 nM) also induced near-complete cytotoxicity by 72 hr, with no visible recovery(**Figure 7-4**). The combination of carboplatin and paclitaxel further increased toxicity, especially at intermediate and high doses with no recovery evident. This suggests OVSAHO cells are sensitive to both agents and that a lower-dose, single-agent strategy may be more informative for this resistance model .

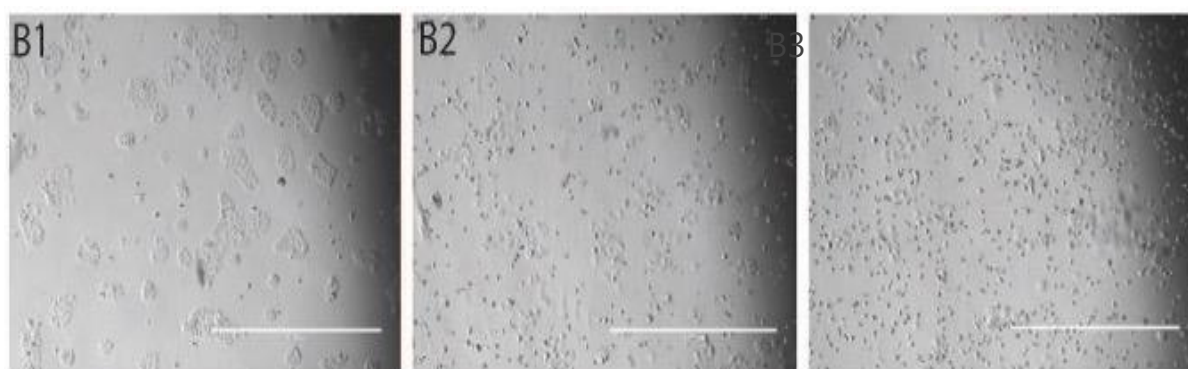


Figure 7-4 OVSAHO cells showing response to escalating carboplatin concentrations

A1 = 6 μ M carboplatin, A2 = 12 μ M carboplatin, A3 = 24 μ M carboplatin. Cells exhibited partial cytotoxicity with evidence of recovery at lower doses and increased cell death at higher concentrations. Images captured under phase-contrast microscopy at $\times 10$ magnification.

7.3.2 IC₅₀ to Guide Development of Resistance Models

Preliminary experiments to generate IC₅₀ values using a resazurin-based viability assay (as per Methods 2.7.1) across a panel of drug concentrations and timepoints. However, the IC₅₀ values generated were inconsistent, often clinically implausible, and demonstrated poor reproducibility, limiting their interpretability (Error! Reference source not found.). Further, the associated dose - response curves frequently failed to meet curve-fitting assumptions, largely due to limited dynamic range and minimal cytotoxicity observed at the concentrations examined (**Figure 7-5, Figure 7-6, Figure 7-7**). Most importantly, the IC₅₀ modelling proved unsuitable for evaluating combination (doublet) chemotherapy regimens (e.g. 20 µM carboplatin with 50 nM paclitaxel), which could not be represented on a log scale and did not reflect the synergistic nature of the treatment. Preliminary data indicated that the combination exerted a greater-than-additive effect, undermining the validity of this method. Given that combination chemotherapy more accurately reflects clinical practice the IC₅₀-based strategy was deemed unsuitable and discontinued. Consequently, a fixed-dose strategy anchored to pharmacokinetic data was adopted for all subsequent work, which better supports clinically interpretable resistance phenotypes.

Cell Line	Treatment	Timepoint	LogIC ₅₀
COV362	Carboplatin (uM)	24 hr	-468.8
COV362	Carboplatin (uM)	48 hr	251.6
COV362	Carboplatin (uM)	72hr	27.2
COV362	Paclitaxel (nM)	24 hr	Undetermined
COV362	Paclitaxel (nM)	48 hr	51.7
COV362	Paclitaxel (nM)	72hr	443.0
COV362	Olaparib (uM)	24 hr	-50.0
COV362	Olaparib (uM)	48 hr	Undetermined
COV362	Olaparib (uM)	72hr	Undetermined
COV362	Chemo Doublet	24 hr	n/a
COV362	Chemo Doublet	48 hr	n/a
COV362	Chemo Doublet	72hr	n/a
OVSAHO	Carboplatin (uM)	24 hr	Undetermined
OVSAHO	Carboplatin (uM)	48 hr	21.8
OVSAHO	Carboplatin (uM)	72hr	32.3
OVSAHO	Paclitaxel (nM)	24 hr	Undetermined
OVSAHO	Paclitaxel (nM)	48 hr	-432.0
OVSAHO	Paclitaxel (nM)	72hr	Undetermined
OVSAHO	Olaparib (uM)	24 hr	Undetermined
OVSAHO	Olaparib (uM)	48 hr	663.0
OVSAHO	Olaparib (uM)	72hr	Undetermined
OVSAHO	Chemo Doublet*	24 hr	n/a
OVSAHO	Chemo Doublet*	48 hr	n/a
OVSAHO	Chemo Doublet*	72hr	n/a
KURAMOCHI	Carboplatin (uM)	24 hr	-409.0

KURAMOCHI	Carboplatin (uM)	48 hr	-285.0
KURAMOCHI	Carboplatin (uM)	72hr	18.4
KURAMOCHI	Paclitaxel (nM)	24 hr	Undetermined
KURAMOCHI	Paclitaxel (nM)	48 hr	52.0
KURAMOCHI	Paclitaxel (nM)	72hr	53.0
KURAMOCHI	Olaparib (uM)	24 hr	Undetermined
KURAMOCHI	Olaparib (uM)	48 hr	64.0
KURAMOCHI	Olaparib (uM)	72hr	20.2
KURAMOCHI	Chemo Doublet	24 hr	n/a
KURAMOCHI	Chemo Doublet	48 hr	n/a
KURAMOCHI	Chemo Doublet	72hr	n/a

Table 7-3 LogIC₅₀ Values Across OC Cell Lines, Treatments, and Timepoints

Log-transformed IC₅₀ values were obtained from non-linear regression analysis of dose–response curves generated using the resazurin reduction assay following 24 hr, 48 hr, and 72 hr drug exposure. IC₅₀ values represent the concentration of drug required to reduce cell viability by 50% relative to untreated (UT) controls. “Undetermined” indicates that an IC₅₀ value could not be calculated due to insufficient curve fitting within the tested range, while “n/a” indicates that the condition was not assessed. ‘Chemo doublet’ refers to combination treatment with carboplatin and paclitaxel.

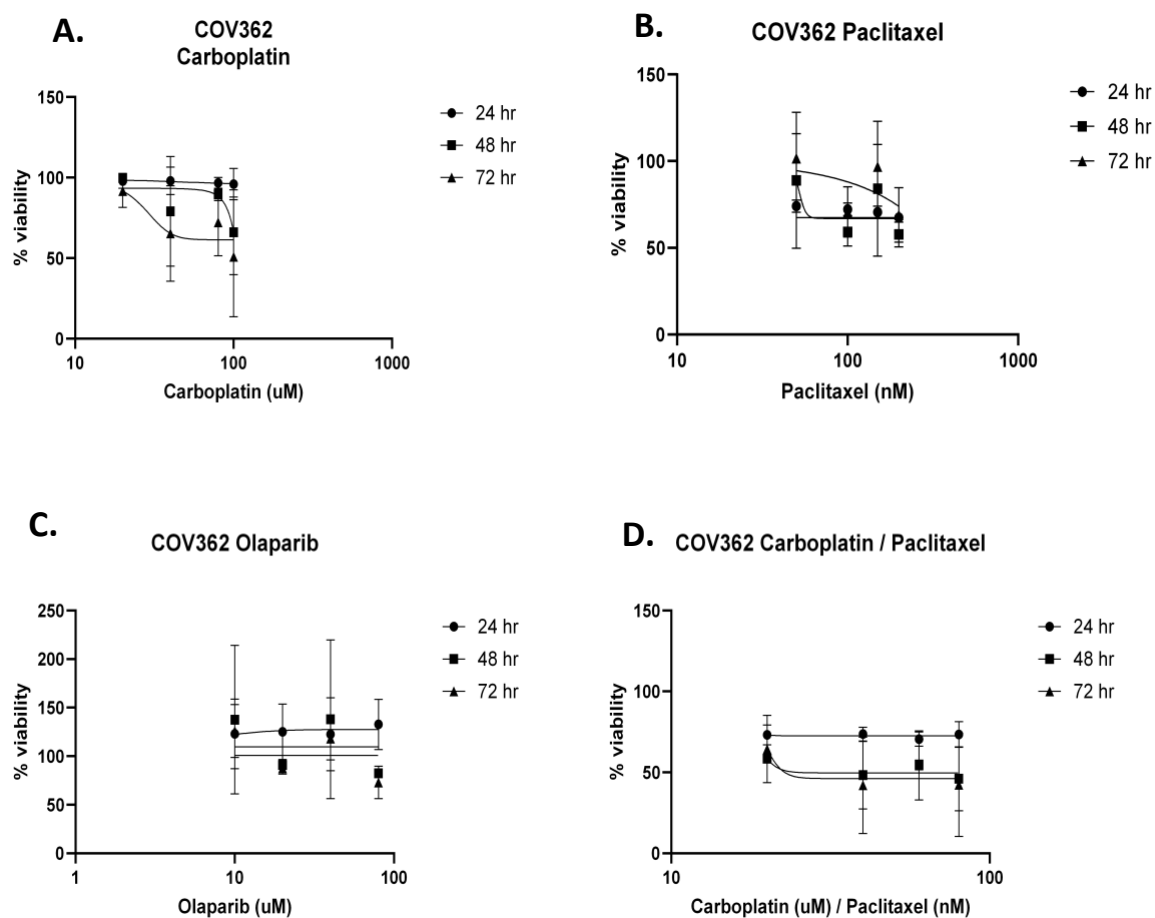


Figure 7-5 Dose–response curves for COV362 across four treatment models

(A) COV362 treated with carboplatin, (B) paclitaxel, (C) olaparib, and (D) carboplatin–paclitaxel combination. Cell viability was assessed using the resazurin reduction assay following 24 hr, 48 hr, and 72 hr drug exposure. Dose–response curves were generated using non-linear regression analysis, with drug concentration plotted on a logarithmic scale to determine IC_{50} values. Data are expressed as percentage cell viability relative to untreated (UT) controls, which were set to 100%. Each data point represents the mean $\pm$ SD from $n = 3$ independent biological replicates performed in triplicate.

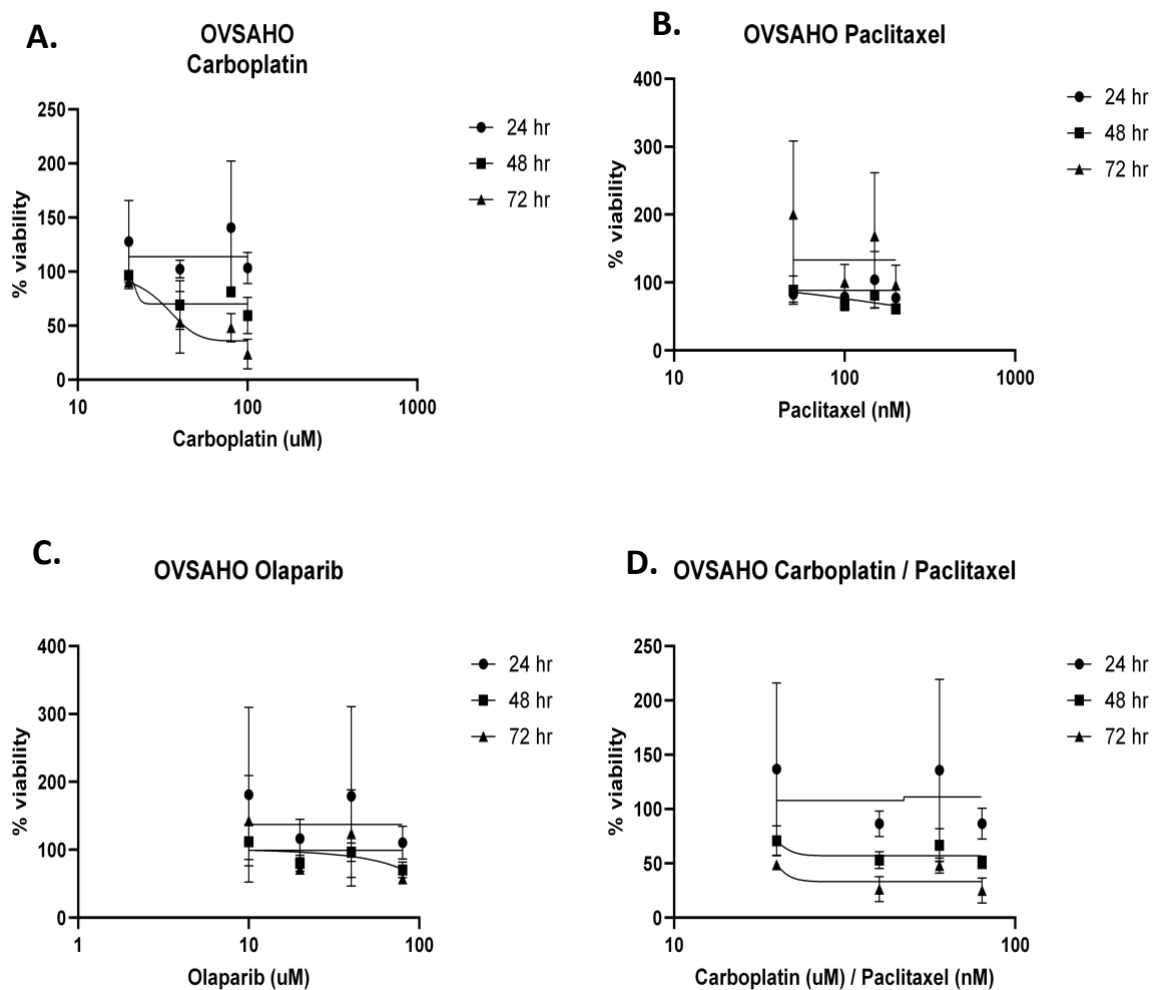


Figure 7-6 Dose–response curves for OVSAHO across four treatment models

(A) OVSAHO treated with carboplatin, (B) paclitaxel, (C) olaparib, and (D) carboplatin–paclitaxel combination. Cell viability was assessed using the resazurin reduction assay following 24 hr, 48 hr, and 72 hr drug exposure. Dose–response curves were generated using non-linear regression analysis, with drug concentration plotted on a logarithmic scale to determine IC_{50} values. Data are expressed as percentage cell viability relative to untreated (UT) controls, which were set to 100%. Each data point represents the mean $\pm$ SD from $n = 3$ independent biological replicates performed in triplicate.

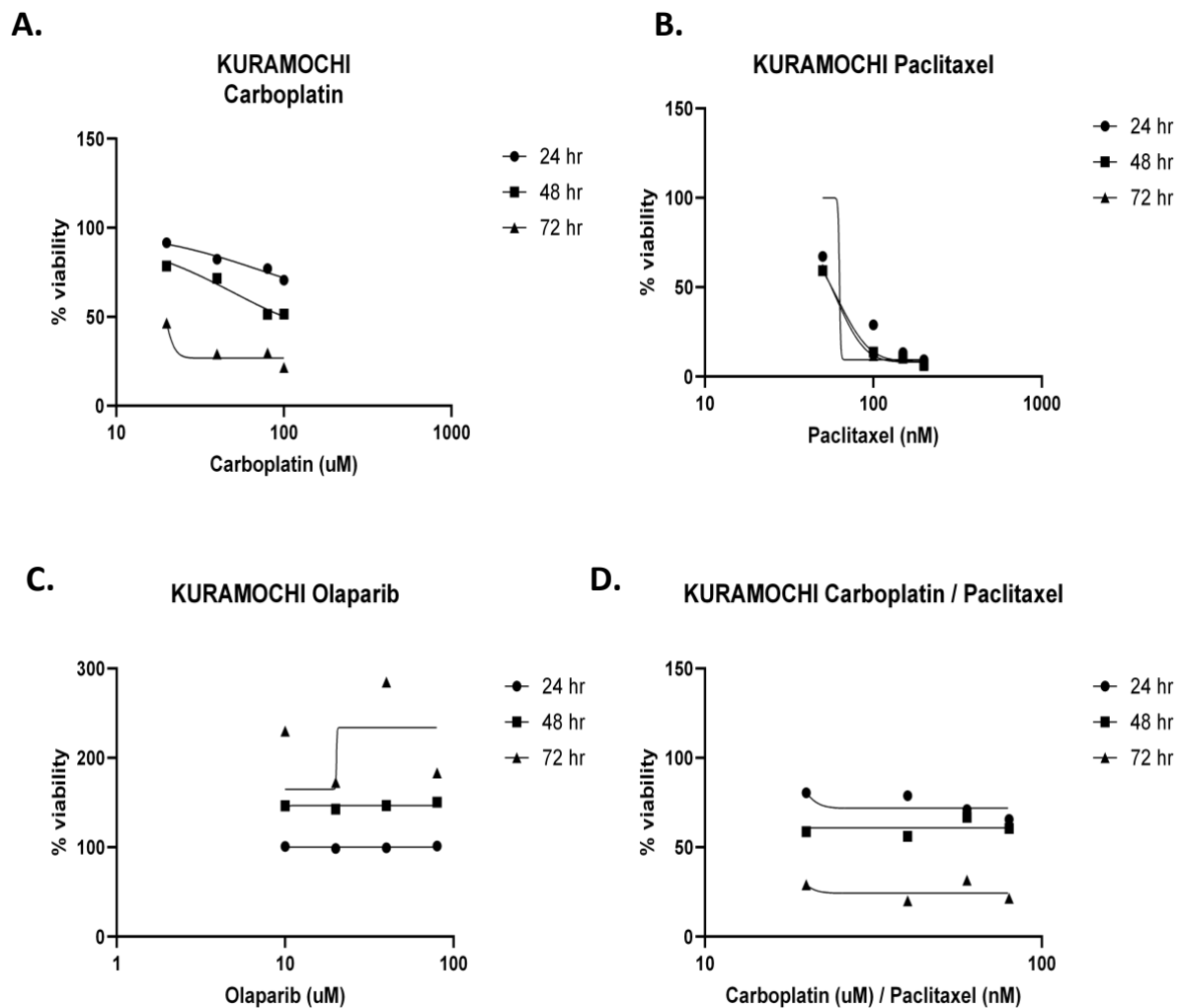


Figure 7-7 Dose–response curves for KURAMOCHI across four treatment models

(A) KURAMOCHI treated with carboplatin, (B) paclitaxel, (C) olaparib, and (D) carboplatin–paclitaxel combination. Cell viability was assessed using the resazurin reduction assay following 24 hr, 48 hr, and 72 hr drug exposure. Dose–response curves were generated using non-linear regression analysis, with drug concentration plotted on a logarithmic scale to determine IC_{50} values at each timepoint. Data are expressed as percentage cell viability relative to untreated (UT) controls, which were set to 100%. Each data point represents the mean $\pm$ SD from $n = 3$ independent biological replicates performed in triplicate.

7.3.3 Transition to a Clinically Relevant Dosage Approach

The strategy was subsequently revised in favour of a 'clinically relevant dosage approach', aligning treatment doses more closely with therapeutic levels observed in clinical practice. Rather than relying on experimentally derived IC₅₀ estimates, fixed concentrations were selected based on published pharmacokinetic data and standard dosing regimens for each agent, as per section 7.3.1.3. Preliminary data supported this strategy, as earlier experiments (as per section 9.1.1) had been modelled around these clinically relevant doses, providing an indication of the concentration range in which cytotoxic effects could be observed, while still allowing for the survival of sub clonal populations. This shift enabled the evaluation of drug effects under conditions that better reflect *in vivo* exposure. The revised approach was particularly important for modelling combination chemotherapy, where synergistic interactions and non-linear dose effects precluded the use of traditional IC₅₀ methods.

Treatment	Target Optimum Dose
Carboplatin	24.24 (μM)
Paclitaxel	58.56 (nM)
Olaparib	16.11 (μM)
Carboplatin / Paclitaxel	24.24 (μM) / 58.56 (nM)

Table 7-4 Dose Optimisation for Treatment Summary

7.3.3.1 Establishing Resistance: Timeline and Observations

COV362, Kuramochi, and OVSAHO OC cell lines were treated over a 14-month period with escalating doses of carboplatin, paclitaxel, olaparib and combination doublet chemotherapy. Treatments were administered approximately every two weeks, when cells reached approximated 70% confluency post recovery. Cells were exposed to chemotherapy agents for 24 hr, and separately to olaparib for 72 hr. For chemotherapy treatments, vehicle control cells were passaged in parallel and exposed to the corresponding drug solvent without active compound. For olaparib, untreated (UT) cells were treated with DMSO to account for solvent effects. Drug doses were escalated gradually based on observed increases in tolerance over time. Initial doses were low to minimise acute cytotoxicity and allow cellular adaptation. For each agent, dose increments were introduced when cells demonstrated recovery and stable growth, as judged qualitatively by microscopic assessment of confluency and morphology. Final target doses were reached over a period of 9–14 months, as summarised in **Appendix 10**.

7.3.3.1.1 Carboplatin

All three cell lines were initially treated with low doses of drug (2–6 μM). COV362 reached 20 μM after 11 months with gradual escalation and manageable recovery. Kuramochi tolerated higher levels, reaching 30 μM after 9 months. OVSAHO demonstrated lower tolerability to treatment, with resistance developing gradually and a final tolerated dose of 12 μM reached after 10 months.

7.3.3.1.2 Paclitaxel

Initial dosing began at 5 nM. Kuramochi and COV362 reached 20 nM after 10 months and showed good recovery between cycles. OVSAHO reached 15 nM after 8 months but required more time to recover, especially beyond 10 nM.

7.3.3.1.3 Olaparib

COV362 and Kuramochi showed early cytotoxic responses at 10 μM , as assessed by morphological changes, but adapted to higher doses over time. By month 13, COV362 reached 60 μM and Kuramochi 100 μM . OVSAHO tolerated olaparib more readily, achieving 100 μM by month 12.

7.3.3.1.4 Chemotherapy Doublet

The doublet regimen was the most cytotoxic. Early treatments at 2 μM carboplatin and 5 nM paclitaxel resulted in high cell loss, as assessed by morphological observation. Drug escalation was slow and conservative. Most lines reached 8/10 or 10/15 μM /nM combinations by late 2024. Cell death and floating debris were frequently observed. Recovery took longer, but stable growth was ultimately achieved across all models.

Cell Line	Drug	Initial Dose	Max. Dose	% Increase	Resistance Achieved (Month)	Stable Growth at Final Dose	Growth Lag / Observations
COV362	Carboplatin	6 μ M	20 μ M	333%	8	Yes	Moderate recovery time; consistent
COV362	Paclitaxel	5 nM	20 nM	400%	8	Yes	Stable after ~10 months
COV362	Olaparib	10 μ M	60 μ M	600%	11	Yes	Slower regrowth beyond 32 μ M
COV362	Carboplatin / Paclitaxel	4 μ M, 10nM	10 μ M / 15 nM	n/a	8	Yes	Most cytotoxic; longer lag phases
Kuramochi	Carboplatin	2 μ M	30 μ M	1500%	8	Yes	Tolerant and fast-growing
Kuramochi	Paclitaxel	15 μ M	20 nM	133%	8	Yes	Minimal lag
Kuramochi	Olaparib	10 μ M	100 μ M	1000%	11	Yes	Consistently tolerant
Kuramochi	Doublet	6 μ M / 15 nM	10 μ M / /15nM	n/a	8	Yes	Well-tolerated after 6 months
OVSAHO	Carboplatin	2 μ M	12 μ M	600%	8	Yes	Slower growth: longer recovery needed
OVSAHO	Paclitaxel	5 μ M	15 nM	300%	8	Yes	Recovery slowed at 10–15 nM
OVSAHO	Olaparib	16 μ M	100 μ M	625%	12	Yes	Prolonged intervals at 40–80 μ M
OVSAHO	Doublet	2 μ M / 10nM	10 μ M / /15nM	n/a	12	Yes	Required longest time to resistance

Table 7-5 Summary of Resistance Induction Across OC Cell Lines and Treatment

7.3.4 Demonstrating Drug Resistance across cell lines

7.3.4.1 Determining suitability of CCK8 Assay

To optimise assay conditions to determine drug resistance, a CCK8 viability assay was performed (as per methods section **2.7.2**) using six seeding densities (500–5000 cells/well) and five timepoints (2, 4, 24, 48, and 72 hr) (**Figure 7-8, Figure 7-9, Figure 7-10**). At earlier timepoints (2 hr and 4 hr), optical density readings at 450 nanometres (OD450) were low and showed limited separation between densities, particularly at ≤ 3000 cells/well. By 24 hr, all three cell lines demonstrated a clear linear relationship between cell number and absorbance, with minimal background noise and good signal resolution across the seeding densities. At 48 and 72 hr, OD450 values increased further, but signal saturation became evident at higher densities (≥ 4000 cells/well), especially in Kuramochi and COV362. OVSAHO showed the lowest baseline activity overall but still displayed linearity by 24 hr. Based on these findings, the 24 hr timepoint was selected for all subsequent .

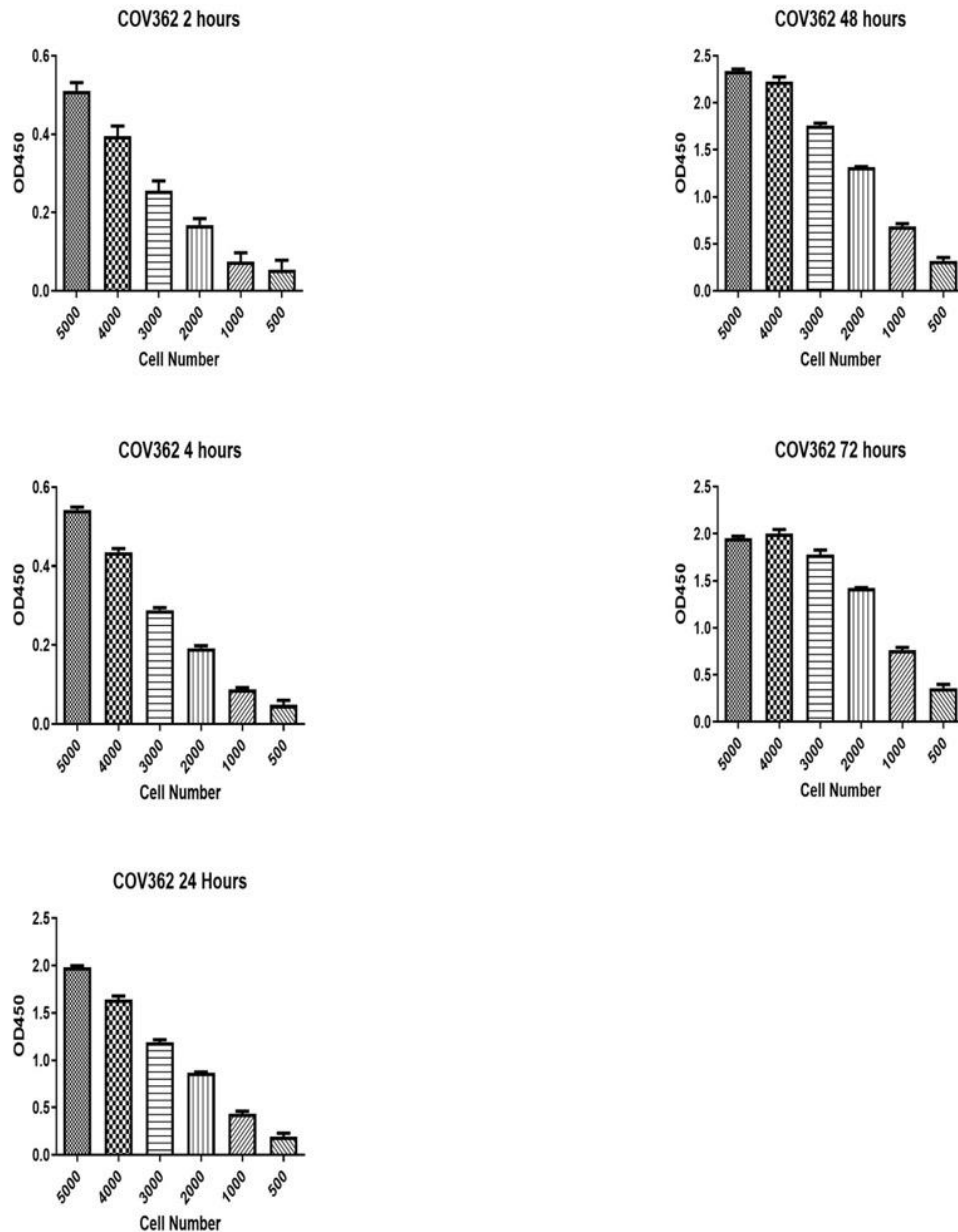


Figure 7-8 OD450 readings at varying cell densities and time points for COV362 cells

CCK8 assay assessing cell viability across six seeding densities (500–5000 cells/well) and five timepoints (2, 4, 24, 48, and 72 hr) to optimise assay conditions for drug resistance experiments. Optical density was measured at 450 nm (OD450). Values represent mean $\pm$ SD of technical triplicates; $n = 3$ biological replicates unless otherwise stated.

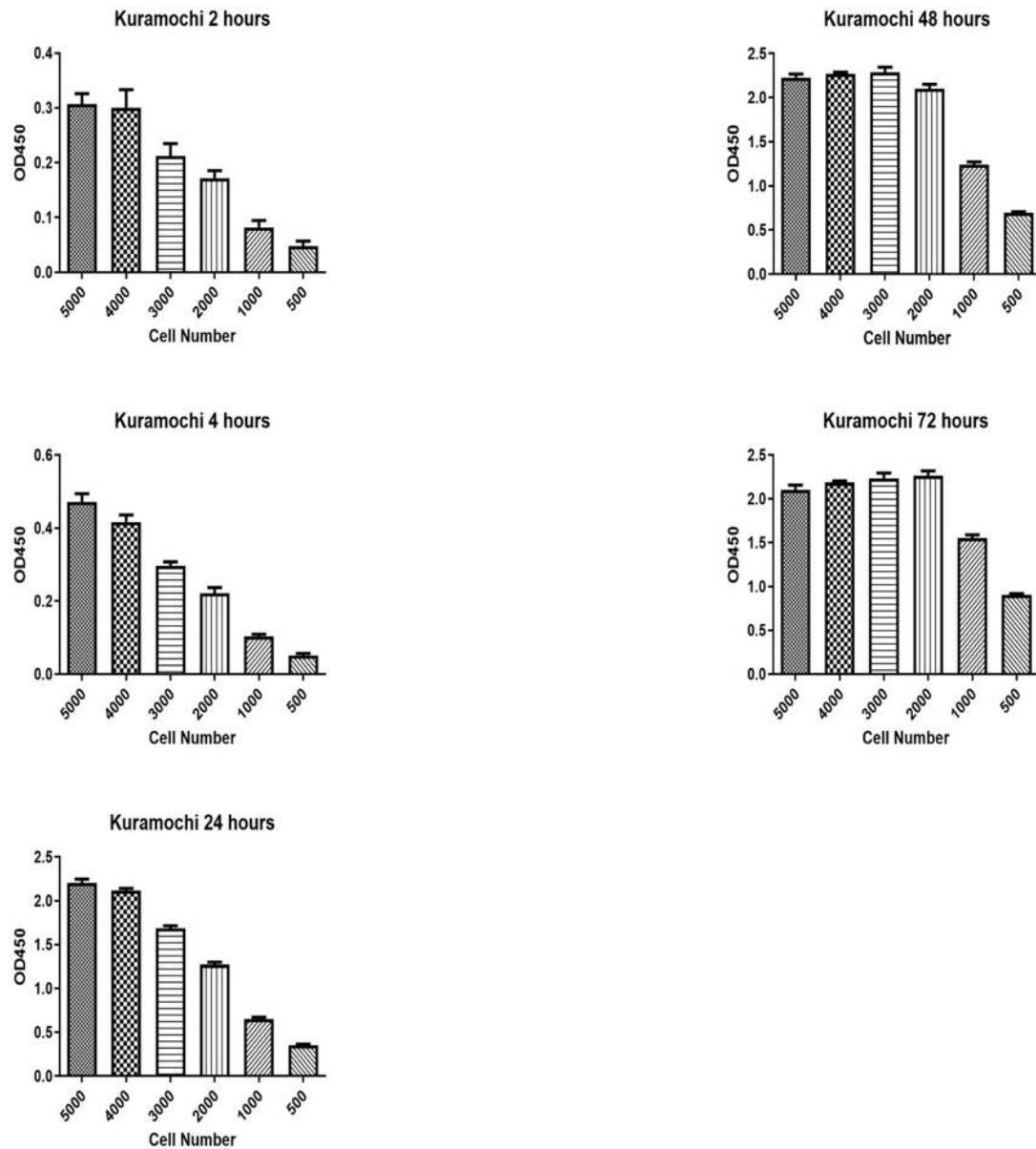


Figure 7-9 OD450 readings at varying cell densities and time points for KURAMOCHI cells

CCK8 assay assessing cell viability across six seeding densities (500–5000 cells/well) and five timepoints (2, 4, 24, 48, and 72 hr) to optimise assay conditions for drug resistance experiments. Optical density was measured at 450 nm (OD450). Values represent mean $\pm$ SD of technical triplicates; $n = 3$ biological replicates unless otherwise stated.

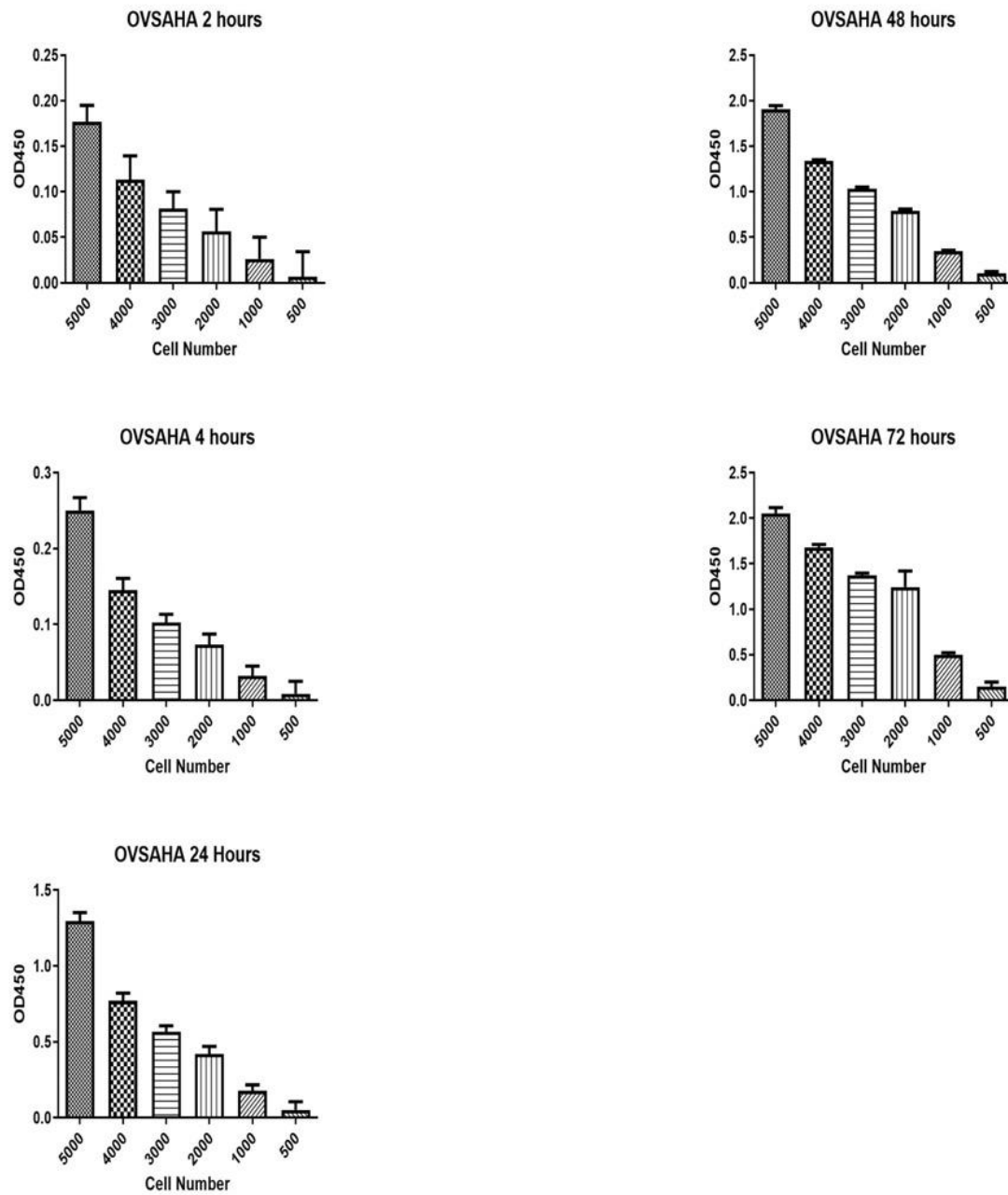


Figure 7-10 OD450 readings at varying cell densities and time points for OVSAHO cells

CCK8 assay assessing cell viability across six seeding densities (500–5000 cells/well) and five timepoints (2, 4, 24, 48, and 72 hr) to optimise assay conditions for drug resistance experiments. Optical density was measured at 450 nm (OD450). Values represent mean $\pm$ SD of technical triplicates; $n = 3$ biological replicates unless otherwise stated.

7.3.4.2 Determination of drug resistance

Cell viability was measured in response to escalating drug concentrations in both parental (UT) and resistant cells using CCK8 assay.

7.3.4.3 COV362 Resistance Model Outcomes

After 10 months of treatment grouped two-way ANOVA demonstrated statistically significant resistance in COV362 cells following treatment with carboplatin, paclitaxel and the carboplatin–paclitaxel doublet (Error! Reference source not found.). For carboplatin, resistant cells showed significantly higher viability across concentrations compared to matched untreated controls ($p < 0.0001$ for treatment effect, interaction and concentration), with a mean viability increase of 10% compared to parental cells. Similarly, paclitaxel-treated resistant cells displayed significantly elevated viability compared to matched untreated controls ($p < 0.0001$), with a mean difference of 11%. The doublet-treated model showed the most marked resistance, with resistant cells exhibiting a 21% higher viability than sensitive cells ($p < 0.0001$), confirming robust adaptation to combination therapy. In contrast, olaparib failed to demonstrate statistically significant resistance; no treatment or interaction effect was observed ($p = 0.5314$ and $p = 0.9768$, respectively).

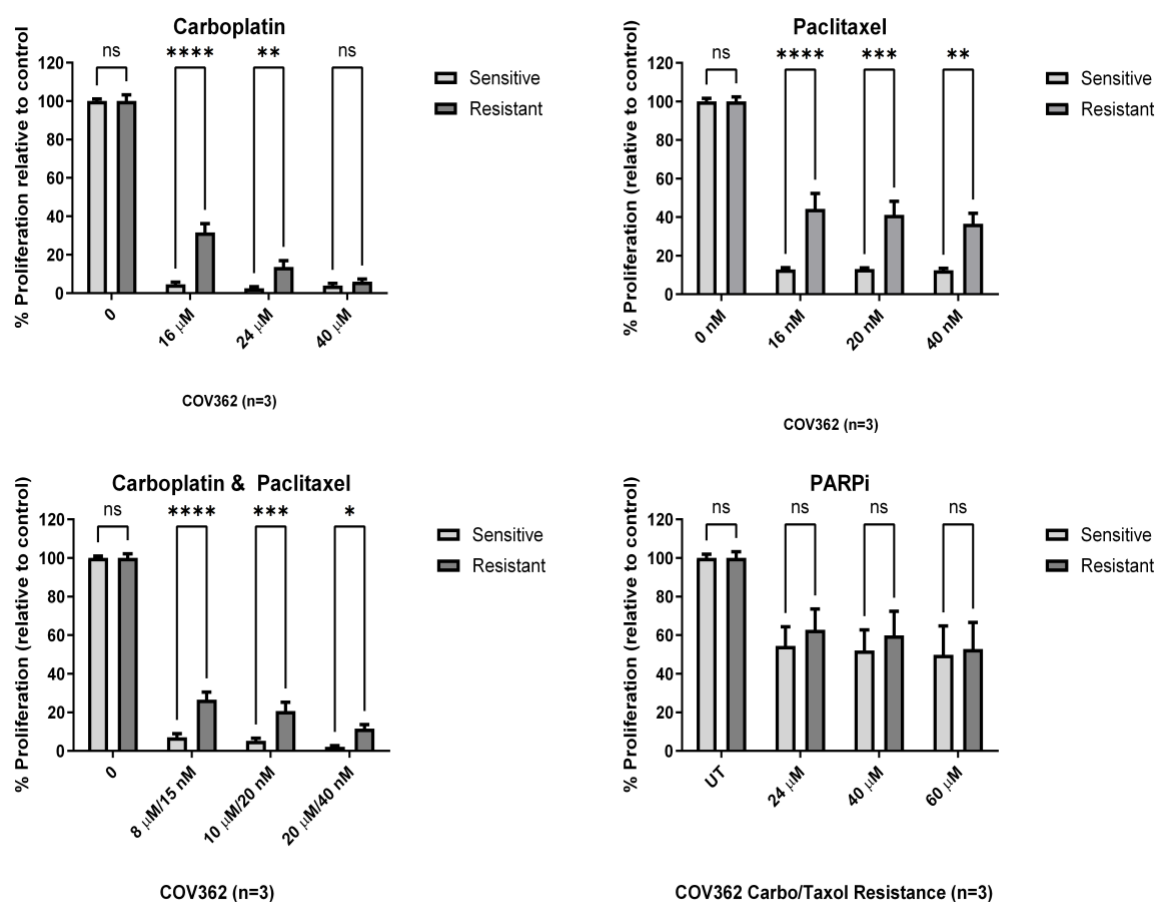


Figure 7-11 Assessment of chemotherapy resistance in *COV362* using CCK8 assay.

Cell viability of chemotherapy-resistant *COV362* models was assessed following treatment with carboplatin, paclitaxel, and the carboplatin–paclitaxel doublet using the CCK8 assay. Viability was compared with matched parental controls to confirm acquisition of resistance. Technical replicates and biological triplicates were analysed (n = 6). Data represent mean $\pm$ SD; statistical significance determined by two-way ANOVA; ((p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), p < 0.0001 (****)).

7.3.4.4 OVSAHO Resistance Model Outcomes

After 10 months of treatment, the OVSAHO cell line grouped two-way ANOVA also revealed various statistically significant resistance following exposure to carboplatin and paclitaxel (**Figure 7-12**). For carboplatin, resistant cells showed a mean viability increase of 6.4% compared to parental cells ($p = 0.0111$), with a significant interaction effect ($p = 0.0090$), confirming a differential response across concentrations. Similarly, paclitaxel-treated resistant cells exhibited significantly greater viability than controls ($p < 0.0001$), with an average increase of 8.9%. In contrast, the carboplatin–paclitaxel doublet model did not reach statistical significance, with no difference in viability between parental and resistant cells ($p = 0.4776$). PARPi treatment again failed to demonstrate significant resistance, with similar viability in both groups ($p = 0.3673$). These results suggest that while OVSAHO cells adapted to single-agent platinum and taxane, resistance to the doublet and PARPi had not fully developed under the experimental conditions tested.

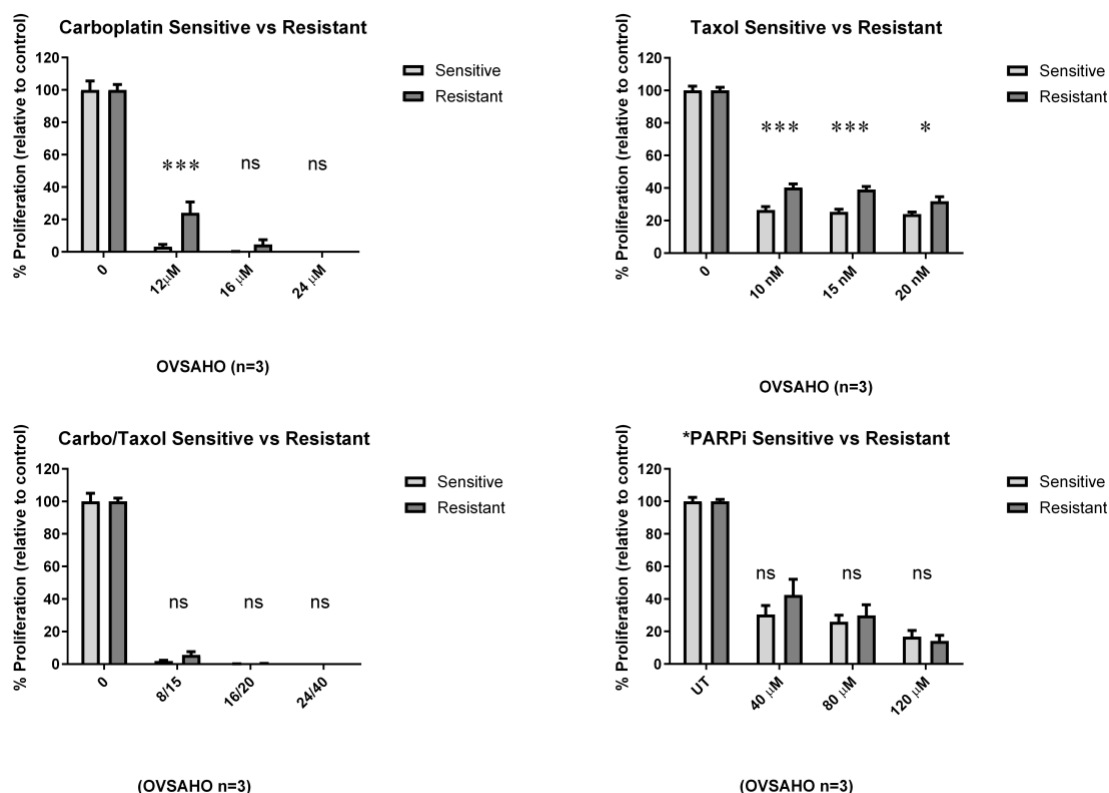


Figure 7-12 Assessment of chemotherapy resistance in OVSAHO using CCK8 assay

Cell viability of chemotherapy-resistant COV362 models was assessed following treatment with carboplatin, paclitaxel, and the carboplatin–paclitaxel doublet using the CCK8 assay. Viability was compared with matched parental controls to confirm acquisition of resistance. Technical replicates and biological triplicates were analysed ($n = 6$). Data represent mean $\pm$ SD; statistical significance determined by two-way ANOVA; ($p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****)).

7.3.4.5 Kuramochi Resistance Model Outcomes

After 10 months of exposure, the Kuramochi cell line demonstrated variable resistance profiles across treatment types (**Figure 7-13**). Significant resistance was confirmed in the chemotherapy-doublet (carboplatin + paclitaxel) model, where a grouped two-way ANOVA revealed a strong main effect of treatment ($p < 0.0001$), with resistant cells showing a higher mean viability (+6.9%, 95% CI: -8.4 to +5.3). For paclitaxel, although the interaction term was not significant, a modest but statistically significant increase in mean viability was observed in resistant cells compared to sensitive (mean difference: +12.0%, $p = 0.048$), suggesting partial adaptation. No evidence of resistance was detected in the carboplatin or olaparib models; differences between resistant and parental cells were small and not statistically significant ($p = 0.31$ and $p = 0.27$, respectively). These findings suggest that Kuramochi cells developed robust resistance to the doublet therapy, with a possible trend toward paclitaxel resistance, but remained largely sensitive to single-agent carboplatin and PARPi.

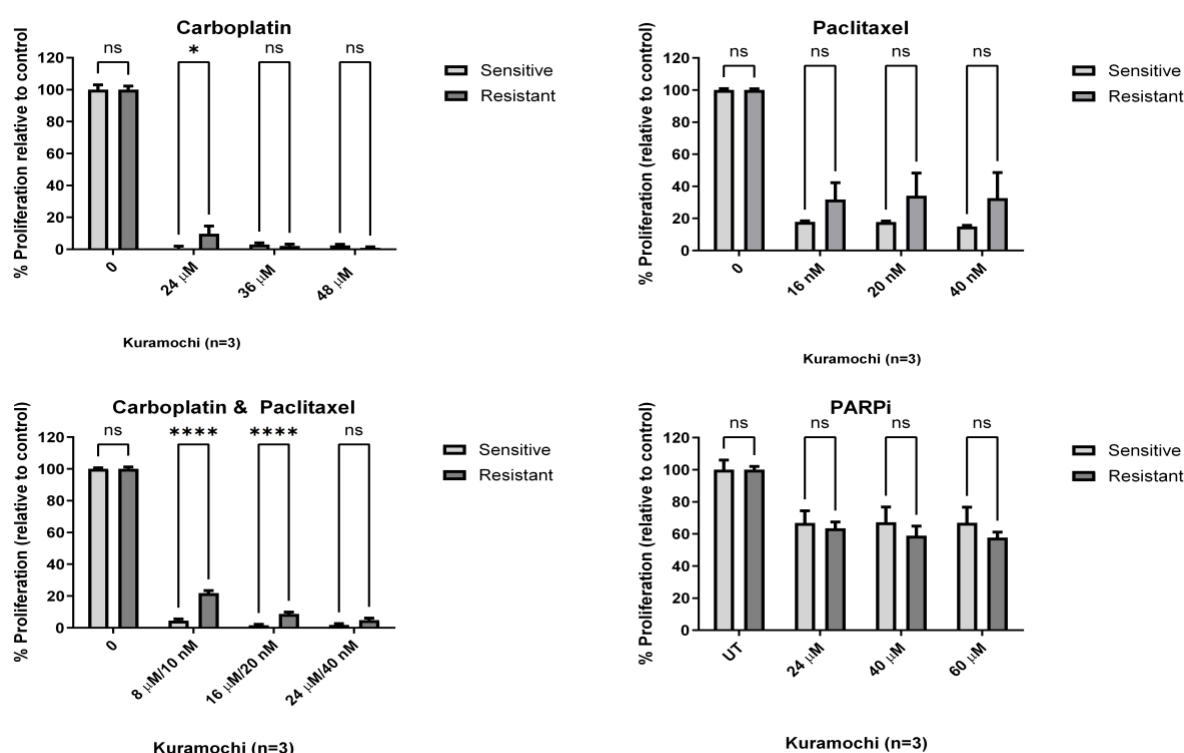


Figure 7-13 Assessment of chemotherapy resistance in KURAMOCHI using CCK8 assay

Cell viability of chemotherapy-resistant COV362 models was assessed following treatment with carboplatin, paclitaxel, and the carboplatin–paclitaxel doublet using the CCK8 assay. Viability was compared with matched parental controls to confirm acquisition of resistance. Technical replicates and biological triplicates were analysed ($n = 6$). Data represent mean $\pm$ SD; statistical significance determined by two-way ANOVA; ($p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****)).

7.3.4.6 Post-Assay Model Considerations

Resistant lines continued to receive a maintenance dose, defined as the highest drug concentration achieved during the resistance protocol, administered every 2–3 weeks to sustain selective pressure. In contrast, models that had not yet reached resistance by this time point remained under active treatment, with gradual dose escalation.

7.3.4.7 Assessment of cellular proliferation

Proliferation assays were conducted approximately five months after the initial viability assessments to reassess, validate and further characterise differences in parental and resistant OC cell line models. At this stage, only the most clinically relevant models were prioritised, including the carboplatin/paclitaxel (doublet) and PARPi resistant lines. Chemotherapy monotherapy models were not included.

7.3.4.8 Proliferation Outcomes in the COV362 Resistance Model

In the Carboplatin/Paclitaxel-resistant COV362 model, proliferation was significantly increased compared to the parental line, with statistical significance observed across all dose levels (low: $p = 0.0001$, medium: $p = 0.0010$, high: $p = 0.0339$) (**Figure 7-14**). A similar pattern was evident in the PARPi-resistant counterpart, where BrdU assay incorporation remained significantly elevated at all doses tested (low: $p < 0.0001$, medium: $p = 0.0007$, high: $p = 0.0358$). These findings indicate that resistance to both chemotherapy and PARPi in COV362 is associated with enhanced proliferative activity.

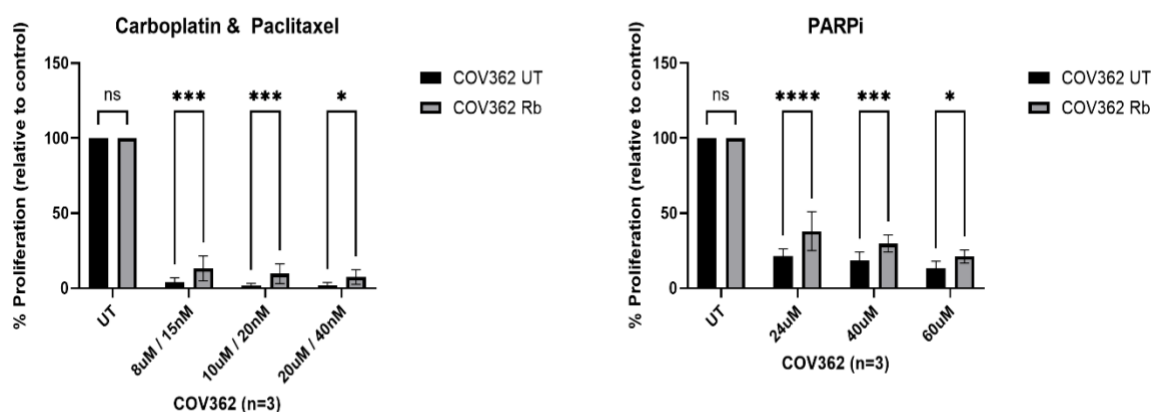


Figure 7-14 BrdU viability of COV362 parental and resistant lines

BrdU assay measuring proliferative activity in parental (UT) and resistant (Rb) COV362 cells following Carboplatin/Paclitaxel or PARPi exposure. Statistical significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****); determined by two-way ANOVA.

7.3.4.9 Proliferation Outcomes in the OVSAHO Resistance Model

Proliferation was modestly but significantly increased in the OVSAHO cells exposed to low-dose doublet chemotherapy ($p = 0.0158$) (**Figure 7-15**). A more pronounced rise in BrdU assay incorporation was observed in the low-dose PARPi-resistant OVSAHO cells ($p < 0.0001$), indicating that both treatment conditions were associated with enhanced proliferative activity, most notably in the PARPi-resistant model.

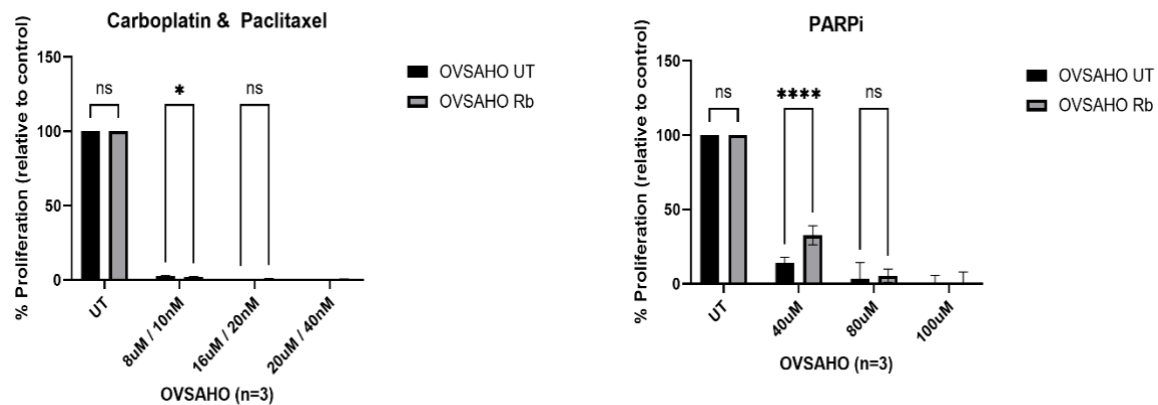


Figure 7-15 Cellular proliferation of OVSAHO parental and resistant lines

BrdU assay measuring proliferative activity in parental (UT) and resistant (Rb) OVSAHO cells following Carboplatin/Paclitaxel or PARPi exposure. Statistical significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****); determined by two-way ANOVA.

7.3.4.10 Proliferation Outcomes in the KURAMOCHI Resistance Model

In the Carboplatin/Paclitaxel-resistant model, a statistically significant increase was observed at low-dose treatment ($p < 0.0001$), which absent at the medium and high doses (**Figure 7-16**). In contrast, the PARPi-resistant Kuramochi cells showed a consistent and highly significant increase in BrdU assay incorporation across all dose levels (low, medium, and high; $p < 0.0001$), representing the largest proliferative shift among all models tested.

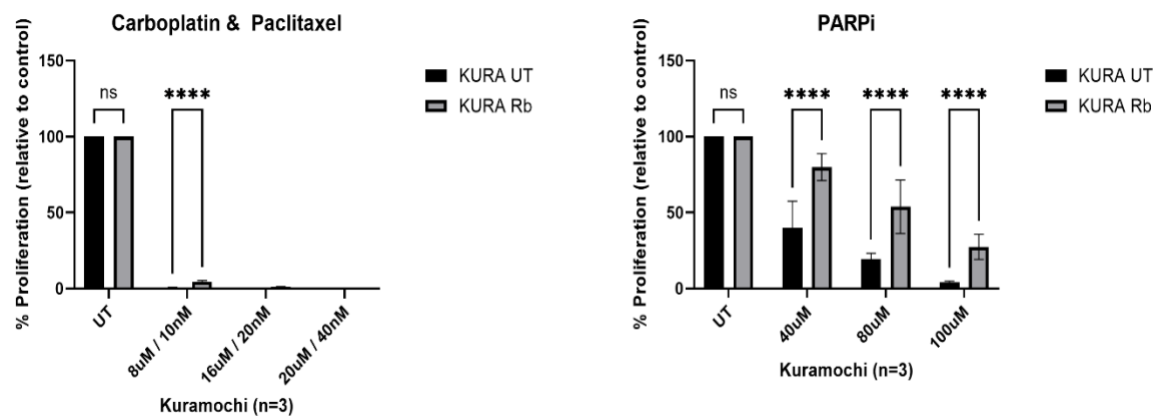


Figure 7-16 BrdU viability of Kuramochi parental and resistant lines

BrdU assay measuring proliferative activity in parental (UT) and resistant (Rb) KURAMOCHI cells following Carboplatin/Paclitaxel or PARPi exposure. Statistical significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****); determined by two-way ANOVA.

7.3.5 STR Profiling of resistant cell lines

Following confirmation of drug resistance, post-sequencing short tandem repeat (STR) profiling was undertaken to authenticate the cell lines. The results indicated that only the COV362 parental and resistant cell line models could be reliably confirmed (**Appendix 9**). In contrast, the other resistant models did not match their expected parental backgrounds and were therefore deemed unsuitable for downstream analysis. On this basis, subsequent investigations were restricted to the authenticated COV362 models.

7.3.6 Assessing PARPi Sensitivity in Chemotherapy Resistant Models

Following initial characterisation of cell viability and proliferation, chemotherapy-resistant models were further evaluated for their response to PARPi treatment. Chemotherapy-resistant COV362 models demonstrated reduced sensitivity to PARPi at low and intermediate concentrations compared with parental cells, indicating partial resistance. At the highest PARPi concentration, a difference in proliferation was observed between resistant and parental models; however, this did not reach statistical significance (**Figure 7-17**).

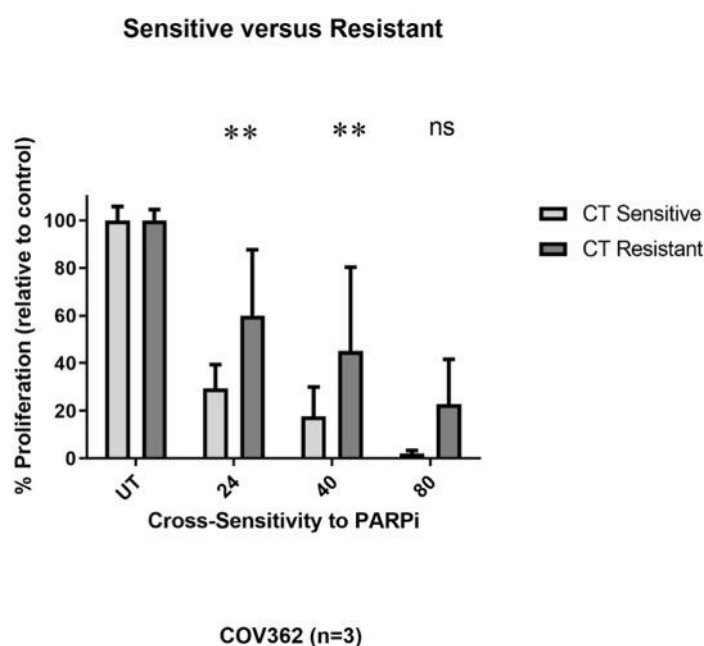


Figure 7-17 Assessing PARPi sensitivity in chemotherapy-resistant models

Chemotherapy-resistant COV362 cells were evaluated for response to PARPi treatment using proliferation assays across a range of concentrations. Proliferation was measured relative to untreated controls to assess potential cross-resistance. Values represent mean $\pm$ SD of technical triplicates; $n = 3$ biological replicates. Statistical significance was determined by two-way ANOVA ($p < 0.05$, (*); $p < 0.01$, (**); $p < 0.001$, (***) ; $p < 0.0001$, (****)).

7.3.7 Stemness Profiling of Resistant OC Models

7.3.7.1 Stemness profiling of chemotherapy-resistant COV362

To determine whether treatment resistance was associated with stem-like characteristics, COV362 chemotherapy- and PARPi-resistant cell line models were analysed for expression of established stemness markers, including *NANOG*, *OCT3/4*, *SOX2*, *NOTCH*, *ADLH1a*, and *KLF4*. A cisplatin-resistant non-small cell lung cancer line (SKMES CisR), previously shown to upregulate stemness pathways (461), was used as a positive control. Unpaired *t*-tests comparing resistant and parental OC cell lines demonstrated significant upregulation of *KLF4* ($p = 0.018$) and *NANOG* ($p = 0.027$) in chemo-resistant cells, while *SOX2* expression was significantly reduced ($p = 0.003$). No significant differences were observed for *ADLH1* ($p = 0.139$), *OCT3/4* ($p = 0.794$), or *NOTCH* (ns) (**Figure 7-18**).

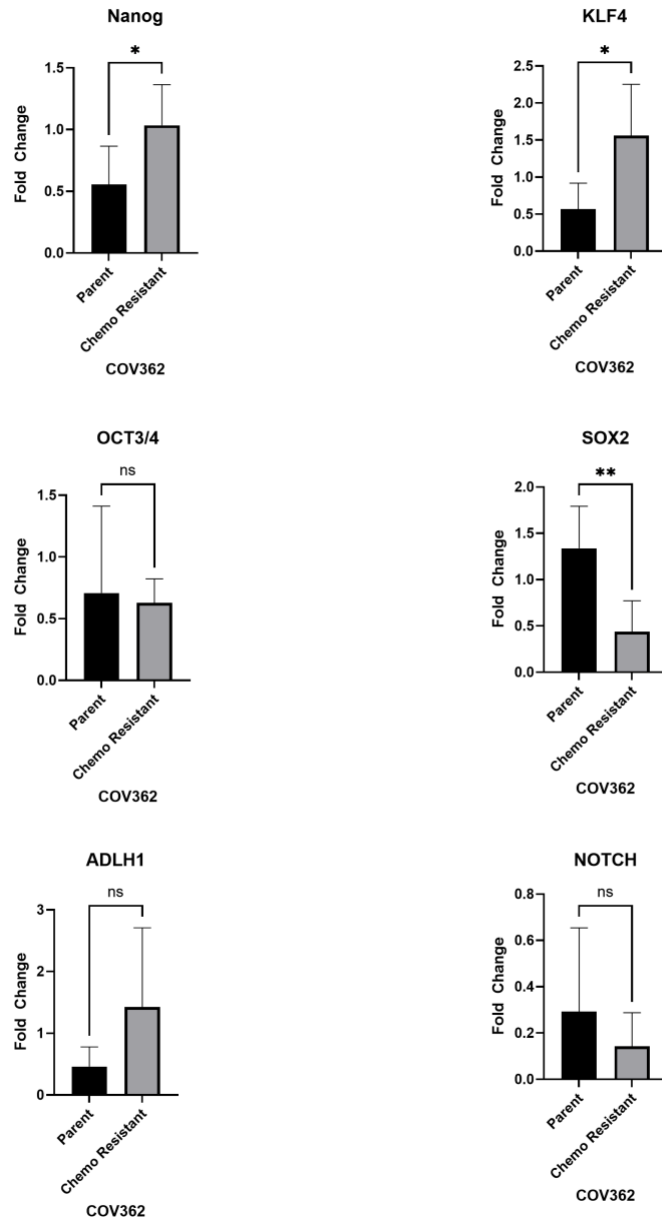


Figure 7-18 Expression of stemness markers in chemotherapy-resistant OC models.

Chemotherapy-resistant COV362 cells were analysed for expression of stemness-associated markers (*NANOG*, *OCT3/4*, *SOX2*, *NOTCH*, *ALDH1A1*, and *KLF4*) by quantitative PCR. Expression levels were normalised to housekeeping gene expression and presented relative to parental COV362 cells. SKMES CisR, a cisplatin-resistant lung cancer cell line, was used as a positive control. Data represent mean $\pm$ SD, with technical replicates and biological triplicates (n = 6). Statistical significance was determined using an unpaired t-test ($p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***; $p < 0.0001$, ****).

7.3.7.2 Stemness Profiling of PARPi - Resistant COV362

To investigate whether PARPi resistance was associated with stem-like characteristics, expression of established stemness markers (*NANOG*, *OCT3/4*, *SOX2*, *NOTCH*, *ADLH1a*, and *KLF4*) was analysed in PARPi-resistant and parental COV362 cell lines. Unpaired *t*-tests demonstrated significant upregulation of *NANOG* ($p = 0.025$) in resistant cells, while no significant differences were observed for *KLF4* ($p = 0.080$), *ADLH1a* ($p = 0.089$), *OCT3/4* ($p = 0.071$), *SOX2* ($p = 0.184$), or *NOTCH* ($p = 0.117$) (Figure 7-19).

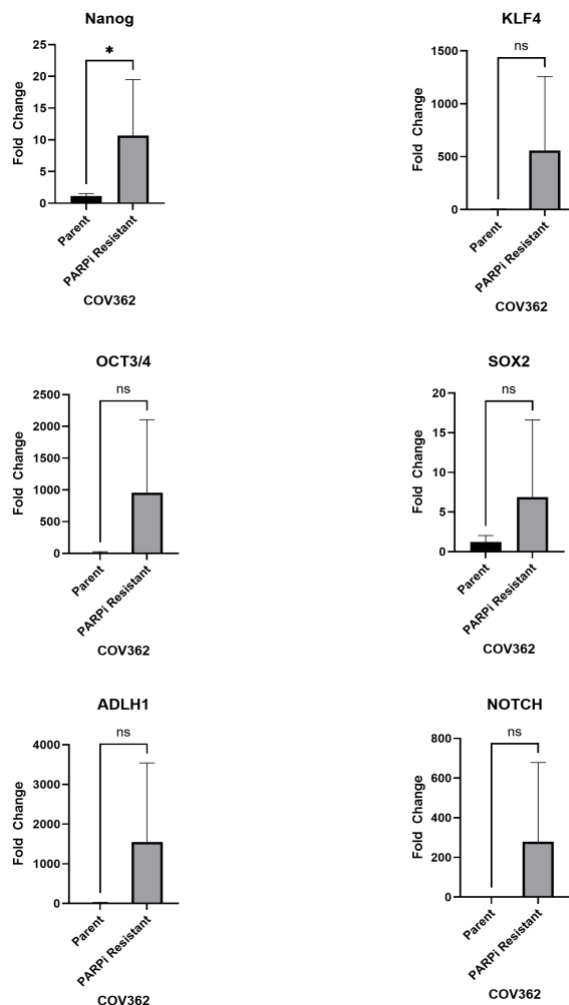


Figure 7-19 Expression of stemness markers in PARPi-resistant OC models.

PARPi-resistant COV362 cells were analysed for expression of stemness-associated markers (*NANOG*, *OCT3/4*, *SOX2*, *NOTCH*, *ADLH1a*, *KLF4*) by quantitative PCR. Expression was compared with parental COV362 cells. SKMES CisR, a cisplatin-resistant lung cancer line, was included as a positive control. Technical replicates and biological triplicates were analysed ($n = 6$). Data represent mean $\pm$ SEM; significance determined by unpaired *t*-test ($p < 0.05$, (*); $p < 0.01$, (**); $p < 0.001$, (***); $p < 0.0001$, (****)).

7.3.8 Influx and Efflux Marker Expression in Resistant OC Models

Given the established role of drug transporters in modulating intracellular drug availability, resistant COV362 models were assessed for changes in influx and efflux marker expression. *CTL1* was evaluated as a representative influx marker, while *ATP7B* was examined as key efflux transporters. In the chemotherapy-resistant model, *CTL1* expression was significantly increased compared with parental cells ($p = 0.027$), whereas no significant difference was observed for efflux transporters ($p = 0.920$). In contrast, in the PARPi-resistant model, *CTL1* expression was significantly reduced ($p = 0.003$), while *ATP7B* levels remained unchanged ($p = 0.979$) (Figure 7-20).

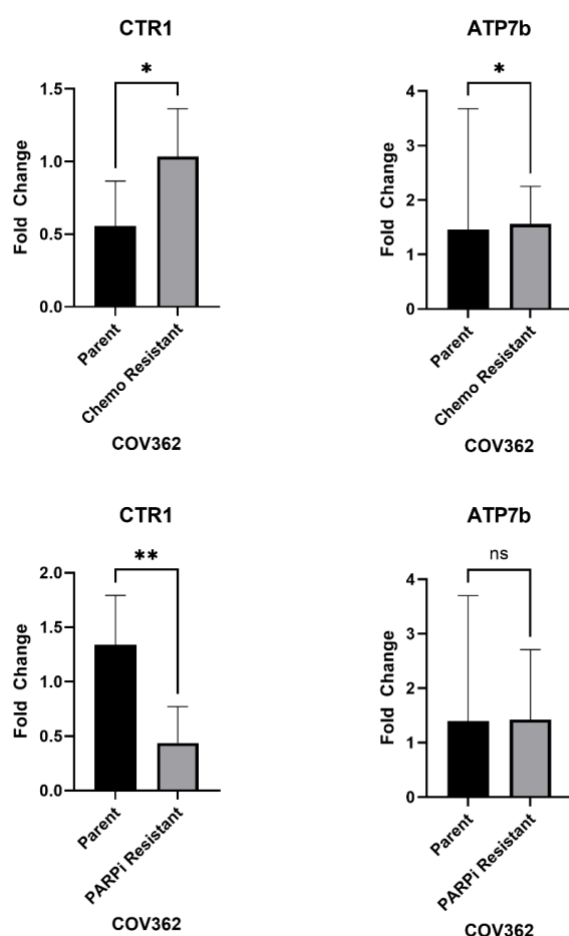


Figure 7-20 Expression of influx and efflux markers in treatment-resistant OC cell lines.

Chemotherapy- and PARPi-resistant COV362 cells were analysed for expression of the influx marker *CTL1* and the efflux transporter *ATP7B* by quantitative PCR. Expression levels were compared with the corresponding parental COV362 line to evaluate potential alterations in drug transport mechanisms associated with resistance. Technical replicates and biological triplicates were analysed ($n = 6$). Data represent mean $\pm$ SEM; significance determined by unpaired t -test ($p < 0.05$, single asterisk; $p < 0.01$, double asterisk; $p < 0.001$, triple asterisk; $p < 0.0001$, four asterisks).

7.4 Discussion

This study focused on developing treatment-resistant OC models using both clinically relevant and high-level laboratory approaches to evaluate resistance mechanisms across therapeutic contexts. Through systematic optimisation of dosing strategies, published pharmacokinetic modelling, and iterative drug exposure, stable resistant populations were generated following repeated cycles of chemotherapy and PARPi treatment. Although cross-contamination was detected in several models during STR authentication, the validated *BRCA1*-mutant COV362 parental and resistant lines were authenticated. These models demonstrated stable resistance to both the chemotherapy doublet and PARPi, providing a robust platform for downstream analysis. Resistance-associated adaptations were further characterised through assessment of stemness marker expression and influx/efflux transporter profiles, offering valuable insights into the cellular mechanisms driving treatment resistance.

7.4.1 Rationale and Limitations in Drug Dosing for Resistance Models

7.4.1.1 Chemotherapy

In COV362, resistance to carboplatin and paclitaxel was observed within 10 months, which was more rapid than the timeframe required for PARPi resistance (14 months). This pattern reflects the higher intrinsic cytotoxicity of platinum–taxane doublets and is consistent with clinical evidence that resistance to chemotherapy often emerges within months in patients with HGSOV (470-472). In contrast, PARPi resistance tends to develop more gradually, with long-term follow-up from the SOLO1 trial demonstrating durable responses extending beyond seven years in a subset of patients receiving maintenance olaparib (59). *In vitro*, chemotherapy resistance was achieved at concentrations approximating those observed in pharmacokinetic studies, lending some reassurance of translational validity. Nonetheless, modelling the combination of carboplatin and paclitaxel presented challenges, as their differing pharmacodynamics and mechanisms limited the generation of conventional sigmoidal dose–response IC_{50} curve. Clinically, patients with *BRCA* CAV generally show improved outcomes on platinum-based chemotherapy, including longer PFS compared with those with *BRCA*-wildtype disease (473).

7.4.1.2 PARPi

The selection of olaparib to represent the PARPi treatment model was more complex than chemotherapy. In the Irish clinical setting, two PARPi agents, olaparib and niraparib, are routinely available (271). Olaparib is indicated specifically for patients with *BRCA1/2* CAV, whereas niraparib was approved by the FDA for ‘all comers’. However, recent revisions to U.S. FDA guidance have led to the withdrawal of niraparib’s approval for use in unselected populations (474). Given these regulatory uncertainties, together with budgetary and practical constraints, olaparib was chosen as the representative PARPi for *in vitro* model development. The development of PARPi resistance

proved more complex than chemotherapy. In COV362, resistance was only demonstrated after 14 months of continuous exposure, and only at concentrations far exceeding clinical relevance (up to 100 μM). Even at supraphysiological levels, complete cell kill was not achieved, preventing the generation of robust sigmoidal IC_{50} curves. Thus, underscoring the difficulty of reconciling pharmacological reality with *in vitro* assay requirements. These findings are consistent with previous reports of olaparib IC_{50} values exceeding 100 μM in OC cell lines (475) highlighting the limited cytotoxic potential of the drug in 2D culture-based systems. Taken together, these results illustrate the pitfalls of supraphysiological dosing, which risks artefactual resistance phenotypes and limits translational validity. It is also important to note that the often-quoted peak plasma concentration of 16 μM is based on early pharmacokinetic data from 400 mg twice-daily dosing (278). The approved clinical dose which is recommended by the NCCP, is 300 mg twice daily (271), which likely results in *lower* steady-state exposure. Clinical trial data support this interpretation. In the SOLO1 study, dose reductions were required in 28.5% of patients, treatment interruptions occurred in over half, and 11.5% discontinued therapy due to toxicity, with only 64.2% of patients remaining on full-dose olaparib at 24 months (476, 477). More recently, the 7-year follow-up of SOLO1 demonstrated durable benefit, with 67.0% of olaparib-treated patients alive at 7 years compared with 46.5% in the placebo arm, and 45.3% vs. 20.6%, respectively, alive and free from subsequent treatment (59). Given this clinical context, the observation that PARPi resistance *in vitro* required prolonged culture and supraphysiological dosing is not unexpected. However, it does represent a fundamental limitation, as extended culture introduces progressive morphological and phenotypic drift, reducing confidence in attributing adaptations specifically to resistance. Importantly, these results emphasise that PARPi resistance *in vitro* appears achievable only at supraphysiological concentrations in the selected cell line panel, underscoring the limitations of such models in fully representing the clinical trajectory of resistance.

7.4.2 Cross-Resistance between Chemotherapy and PARPi

The observation of partial, dose-dependent cross-resistance between platinum-based chemotherapy and PARPi in the COV362 model reflects the mechanistic overlap between these agents, both of which target DNA damage repair pathways (478). This finding supports clinical observations that prior platinum exposure can reduce subsequent PARPi responsiveness in BRCA-mutant OC (479) and vice versa (480). The results support existing evidence that resistance mechanisms acquired under platinum pressure, such as restoration of homologous recombination or enhanced DNA repair capacity, may concurrently diminish PARPi sensitivity (481). These findings reinforce the clinical importance of treatment sequencing in BRCA-mutant OC and underscore the need for biomarkers capable of identifying patients likely to retain PARPi sensitivity following treatment with platinum-

based therapy.

7.4.3 Stemness-Associated Adaptations in Resistance

Upregulation of NANOG and KLF4 in chemotherapy-resistant COV362 models suggests the emergence of stem-like features during resistance development, supporting the concept that resistant subclones adopt less differentiated states to withstand drug exposure. In PARPi-resistant lines, more modest alterations in stemness markers may reflect early adaptive reprogramming rather than a full stem-like transition. These findings are consistent with recent evidence that *KLF5* enhances stemness and contributes to PARPi resistance in OC by regulating *Vimentin* expression, where inhibition of *KLF5* reversed stem-like characteristics and resensitised resistant cells to PARPi. Although variability between replicates limited statistical significance, the current data collectively indicate a potential role for stemness pathways in sustaining resistance(253). Although variability between replicates limited statistical significance, these findings collectively indicate a potential role for stemness pathways in sustaining resistance. Validation through functional assays in expanded models will be important to confirm their biological relevance.

7.4.4 Drug Transporter Dynamics in Resistant Models

Altered expression of drug transporters in resistant COV362 models highlights the role of membrane dynamics in modulating intracellular drug exposure. Increased *CTL1* and *ATP7B* expression in chemotherapy-resistant cells may enhance both drug uptake and efflux capacity, supporting a coordinated adaptation to maintain intracellular homeostasis under cytotoxic stress. In contrast, *CTL1* expression was reduced and *ATP7B* remained unchanged in PARPi-resistant cells, suggesting that transporter-mediated mechanisms are less relevant to PARPi adaptation. This aligns with previous reports that influx and efflux transporters play a minimal role in PARPi resistance (482), whereas their involvement in platinum and taxane resistance is well established (230, 461). Together, these findings support the presence of distinct, treatment-specific adaptive responses and underscore the multifactorial nature of drug resistance in *BRCA*-mutant OC.

7.4.5 Methodological Considerations in assays used to determine resistance

Developing resistance models presented several methodological challenges. A first limitation related to dosing strategy. While clinical pharmacokinetic data provided a framework for estimating achievable plasma concentrations, applying these to an *in vitro* systems proved difficult. The chemotherapy doublet posed challenges, as carboplatin and paclitaxel act through distinct pharmacodynamics and mechanisms, preventing the derivation of a unified IC₅₀ curve. In the PARPi models, even at supraphysiological concentrations, complete cell kill was not achieved, precluding conventional sigmoidal dose–response fitting. Together, these constraints required alternative dosing strategies that prioritised feasibility over strict pharmacological fidelity.

A second issue was the duration of culture required to induce resistance. Chemotherapy resistance emerged within 10 months in *BRCA1/2* mutant models but required longer exposure in the wildtype background, whereas PARPi resistance only became demonstrable after 14 months. These extended culture times introduce a risk of phenotypic drift, including altered growth kinetics and morphology, which may confound interpretation of resistance-specific adaptations (267, 268, 437).

The mode of drug administration also carried implications for model validity. Continuous exposure was selected to approximate steady-state plasma levels, but this approach overlooks important clinical realities such as pulsed dosing, treatment breaks, and dose reductions, all of which influence resistance trajectories in patients. In addition, resistance was modelled in a mixed cell population rather than through isolation of individual resistant clones, which may have masked clonal heterogeneity and limited insight into specific adaptive mechanism. Consequently, the models may have captured only a narrow spectrum of the mechanisms that evolve *in vivo*.

Finally, the simplified context of cell culture omits critical factors such as stromal interactions, immune surveillance, and intra-tumour heterogeneity, all of which play central roles in shaping resistance. In this study, proliferation and viability assays were primarily used to confirm the development of resistance; however, complementary functional assays such as apoptosis or DNA damage response analysis could provide further insight into the underlying mechanisms. Thus, while these assays successfully demonstrated that resistance can be induced *in vitro*, they should be regarded as complementary systems best suited to exploring molecular mechanisms, rather than as accurate surrogates of clinical resistance dynamics. For example, immune cells, stromal cells and blood vessels are absent in cell culture. Additionally, there is concern that cell lines may lose their characteristic and genetic features after prolonged culture (270).

7.4.6 Challenges Encountered and Limitations

Several challenges were encountered during the development of resistant cell line models, most notably microbial contamination during prolonged culture. Episodes of bacterial, fungal, and mycoplasma growth occasionally occurred over the 14-month period. While some infections were successfully treated, others required the affected line to be discarded and re-established from the earliest cryopreserved passage, highlighting the importance of maintaining reliable frozen stocks. Control and treatment flasks were managed identically to ensure accurate interpretation of drug response, and treatment was resumed at the dose reached by the most recent uncontaminated passage. A further and well-recognised challenge in cell culture research is the risk of cross-contamination between cell lines. This issue has been documented for decades, particularly in highly proliferative cancer cell lines that can overgrow co-cultured or neighbouring populations, as exemplified by historical HeLa cell contamination, which is still an issue in modern research (483,

484). In this study, the extended culture times required for the development of PARPi-resistant models likely compounded this risk, as prolonged drug exposure necessitates continuous maintenance over many months. STR profiling ultimately revealed cross-contamination among several resistant models, with only the COV362 parental and resistant lines confirmed as authentic. Although the contaminating profile did not match HeLa-derived lines, the findings suggested that COV362 cells had overgrown or displaced other cultures during long-term maintenance. This may have arisen from inadvertent human error during handling or may reflect the inherently aggressive nature of this OC line - or a combination of both. Taken together, these challenges highlight the inherent complexities of developing resistant models under long-term drug selection and emphasise the necessity for stringent contamination control, periodic authentication, and careful management of passage numbers to preserve model integrity.

7.5 Future Work

Future work should aim to expand the repertoire of resistant OC models across distinct *BRCA* backgrounds, enabling direct comparison of resistance trajectories to platinum-based chemotherapy and PARPi. A broader set of validated models would provide a stronger foundation for identifying genotype-specific patterns of resistance.

Beyond model generation, mechanistic studies will be important to clarify the role of stemness markers and drug transporters in resistance. This will involve moving beyond targeted RNA-level profiling to protein-level validation, incorporating techniques such as western blotting, ELISA, and immunofluorescence. This will strengthen the capacity to interrogate molecular mechanisms directly and provide a more comprehensive picture of how resistance emerges and is sustained.

Future directions should also include the use of targeted perturbation strategies, such as siRNA knockdown or CRISPR-based editing, to determine whether pathways implicated in stemness, or efflux play a causal role in the resistant phenotype. Integrating these mechanistic studies with broader omics approaches, including transcriptomic and epigenomic profiling, would generate a multidimensional understanding of resistance biology.

Ultimately, complementing *in vitro* experiments with more complex patient-derived models, such as xenografts or organoids, will be critical for validating findings in a clinically relevant context. Together, these approaches would advance both technical skills and biological insight, bridging the gap between laboratory observations and translational application in OC.

circRNA profiling in Treatment Resistant OC

Chapter Eight

8 circRNA profiling in Treatment Resistant OC

8.1 Introduction

Treatment resistance remains a major clinical challenge in OC, with the majority of patients eventually relapsing despite initial responsiveness to platinum-based chemotherapy. Acquired resistance limits the long-term efficacy of both cytotoxic agents and PARPi, and the underlying mechanisms remain only partly understood. Elucidating these mechanisms is critical to improving patient outcomes and informing the development of more durable therapeutic strategies. In Chapter 7, resistant OC cell line models were generated to provide an in vitro platform for studying resistance. While these models confirmed phenotypic differences between sensitive and resistant cell lines, further molecular profiling is required to identify the regulatory features that underpin resistance and to define potential therapeutic targets.

As described in Chapter 6, circRNAs represent an emerging class of non-coding RNAs with diverse roles in post-transcriptional gene regulation (485). Despite their emerging importance, the circRNA landscape in resistant OC remains poorly characterised. While there has been a recent increase in studies examining circRNAs in OC (177, 181, 182, 214, 486-490), many rely on models that do not reflect clinical practice, such as the use of cisplatin instead of the more broadly used carboplatin, or employ cell lines that are recognised as poor representatives of HGSOC (184). Advances in array-based technologies, such as circRNA microarrays, now enable the systematic interrogation of circRNA expression in cell lines and tumour tissues.

By leveraging the resistant cell lines established in Chapter 7, this chapter applies high throughput circRNA profiling to uncover expression changes specifically associated with resistance. This study utilised two resistance models in a clinically relevant HGSOC cell line, COV362 (*BRCA1* mutant), using a carboplatin/paclitaxel (C/T) and Olaparib.

8.2 Aims and Objectives

8.2.1 General Aims

This chapter focuses on profiling circRNA expression in treatment-resistant OC models. It aims to identify circRNAs that are differentially expressed between resistant and parental cell lines and to evaluate their potential as biomarkers of therapeutic resistance.

8.2.2 Hypothesis

The hypothesis is that circRNA expression profiles differ between treatment-sensitive and treatment-resistant OC cell lines, and that these differences highlight circRNAs with biomarker or potential functional relevance in resistance.

8.2.3 Objectives

The objectives of this chapter were to:

- Determine the circRNA profile in resistant and parental OC cell lines
- Characterise the circRNA landscape by integrating differential expression and functional analyses, and identify candidate biomarkers for subsequent validation
- Validate candidate circRNAs across a panel of OC cell lines

8.3 Results

8.3.1 Visualisation of circRNA Expression Patterns in Resistant Models

8.3.1.1 Hierarchical Clustering Heatmaps

OC cell lines underwent circRNA profiling as per methods 2.11. Unsupervised hierarchical clustering heatmaps were generated to compare circRNA between untreated parental *COV362* cells (vehicle control) and their chemo-resistant and PARPi-resistant sublines. In the untreated vs. chemo-resistant comparison, the clustering heatmap showed that untreated cells grouped together on one major branch, while chemo-resistant replicates clustered on the opposite branch (**Figure 8-1**). This separation was accompanied by blocks of circRNAs with consistently higher or lower expression in the resistant cells, indicating possible transcriptomic reprogramming following chemotherapy exposure. In the untreated vs. PARPi-resistant comparison, untreated and resistant cells also segregated (**Figure 8-2**), although the clustering appeared less distinct. Heatmap colour patterns suggested greater heterogeneity within the PARPi-resistant group, with smaller subsets of circRNAs showing consistent directional shifts. Across both comparisons, subsets of circRNAs consistently differed between untreated and resistant samples. These circRNAs were prioritised for downstream enrichment and pathway analyses to identify resistance-associated signatures.

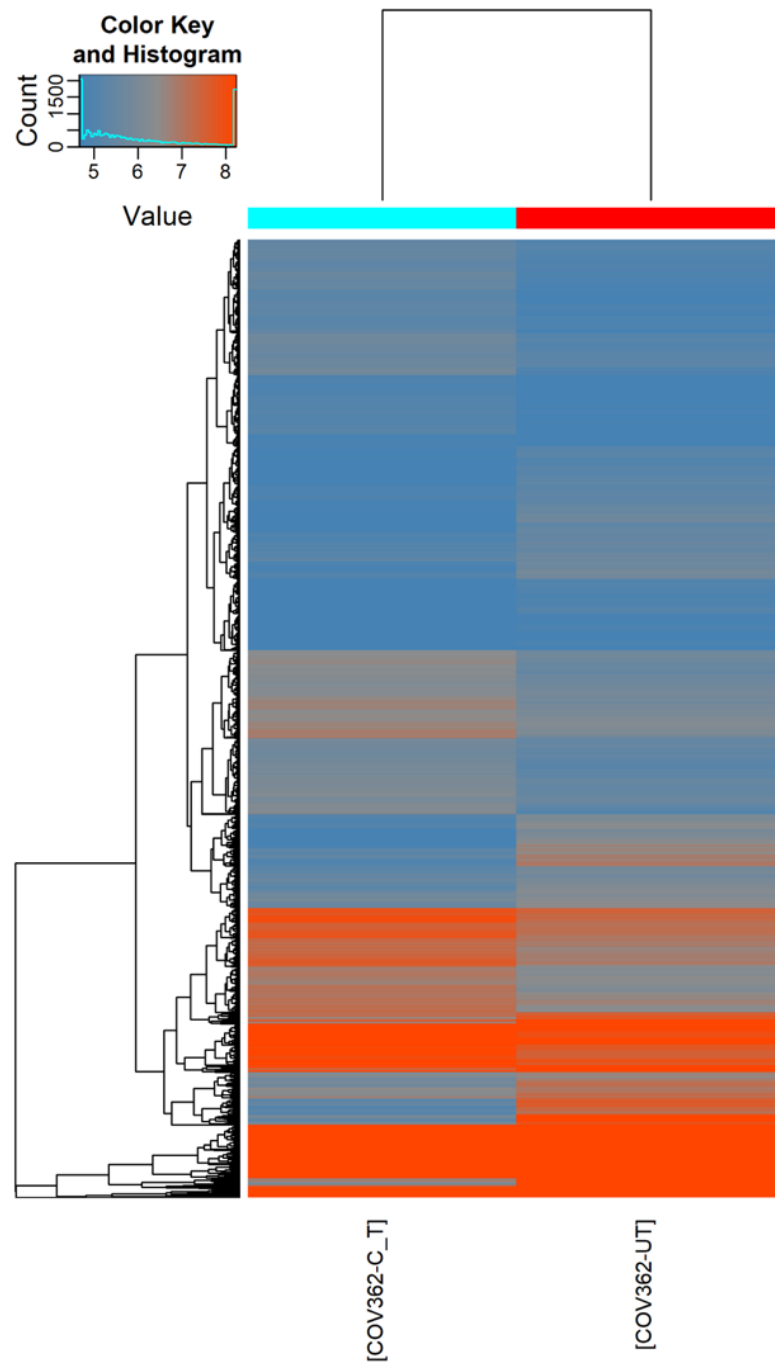


Figure 8-1 Heatmap of circRNA expression chemo-resistant vs. UT

Unsupervised hierarchical clustering of circRNAs in COV362 model (n=3). Columns represent samples; rows represent circRNAs (row-wise z-scores). Dendrograms show clear segregation of untreated and chemo-resistant groups with coherent expression blocks distinguishing conditions.

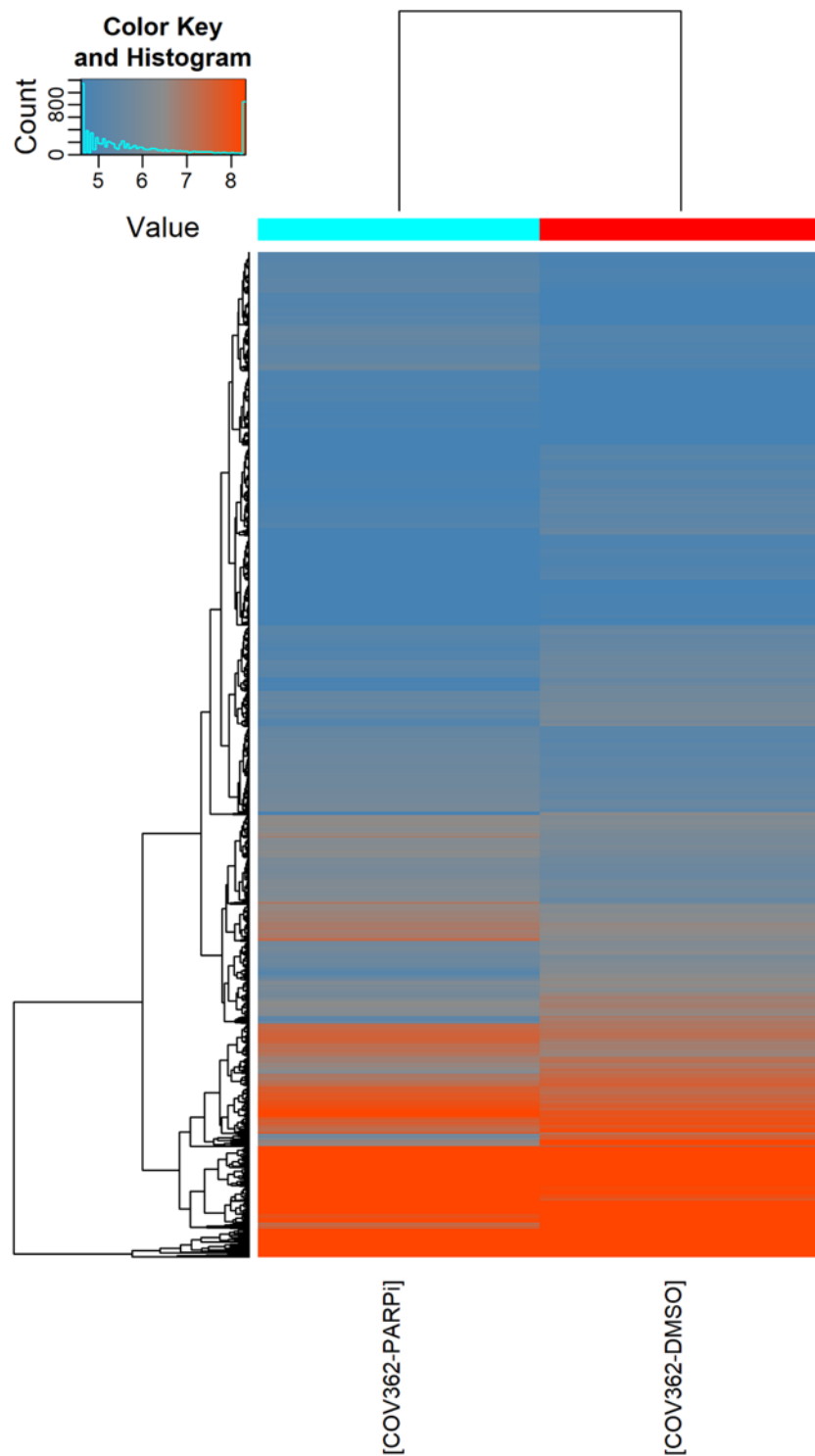


Figure 8-2 Heatmap of circRNA expression PARPi-resistant vs. UT.

Samples segregate by condition, with modest within-group heterogeneity observed in the PARPi-resistant cells and smaller circRNA modules marking the resistant state. The DMSO-treated parental *COV362* line served as the untreated (vehicle control) reference for comparison.

8.3.1.2 Scatter Plots

Scatter plots were generated to visualise circRNA expression changes between untreated and resistant COV362 cells. In the chemotherapy-resistant comparison, circRNA expression showed a Pearson correlation of 0.9061 between untreated and resistant states, indicating broad conservation of global expression (**Figure 8-3**). Each point represents a circRNA in the COV362 model. Red points denote upregulated circRNAs (n = 3,385), blue denote downregulated circRNAs (n = 5,027), and grey indicate non-differentially expressed circRNAs (n = 4,611). A subset of circRNAs deviated from the diagonal, with upregulated circRNAs in untreated samples forming a dispersed, sunburst-like pattern, while downregulated circRNAs clustered more closely with the non-differentially expressed population. This suggests that chemotherapy resistance is associated with a focused subset of circRNA alterations superimposed on a largely stable background. In the PARPi-resistant comparison, a higher Pearson correlation of 0.9776 was observed, reflecting greater overall similarity between untreated and resistant states (**Figure 8-4**). Each point represents a circRNA in the COV362 model. Red points denote upregulated circRNAs (n = 1,898), blue denote downregulated circRNAs (n = 2,178), and grey indicate non-differentially expressed circRNAs (n = 8,947). Despite the high overall correlation, a small subset of circRNAs was upregulated, whereas downregulated circRNAs remained more closely aligned with the non-differentially expressed group. These patterns indicate that PARPi resistance involves subtle but heterogeneous transcriptional changes, with fewer circRNAs showing strong divergence compared with the chemotherapy-resistant model.

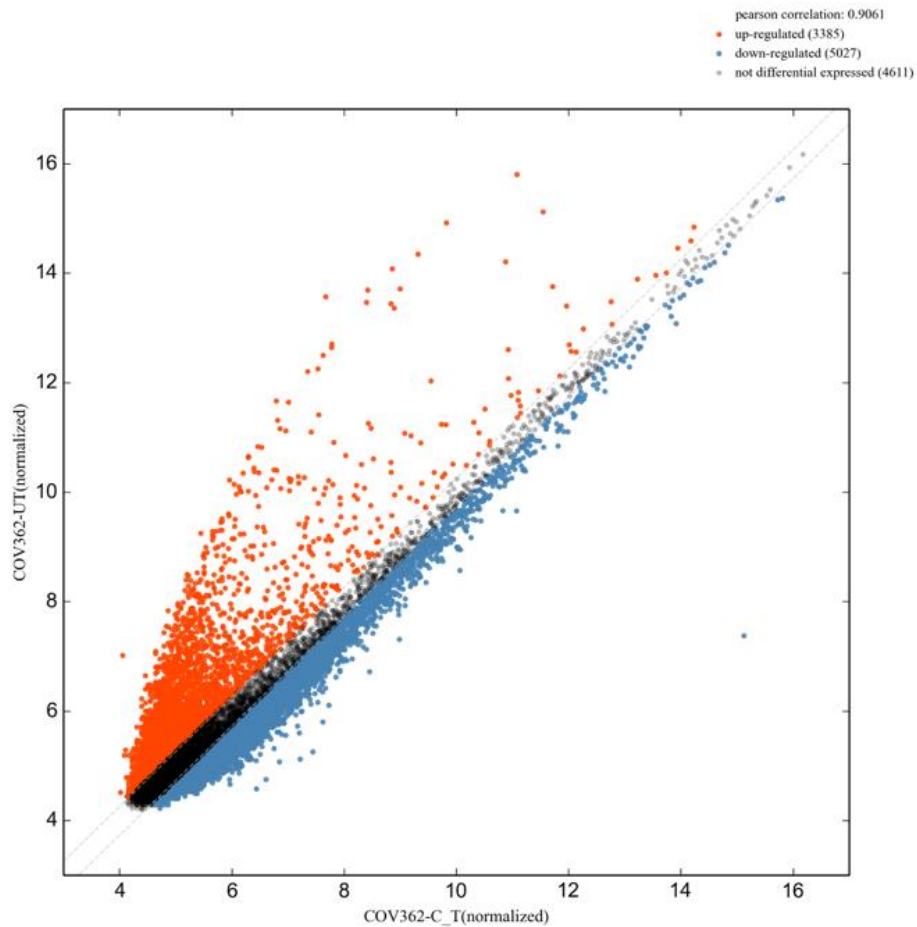


Figure 8-3 Scatter plot of circRNA expression in chemo-resistant vs. UT

Each point represents an individual circRNA, plotted according to relative expression in the two conditions. Data shown represent the mean expression values derived from three biological replicates ($n = 3$). The distribution illustrates overall conservation of circRNA expression between parental and resistant states, with a subset of circRNAs showing divergence consistent with chemotherapy-associated alterations.

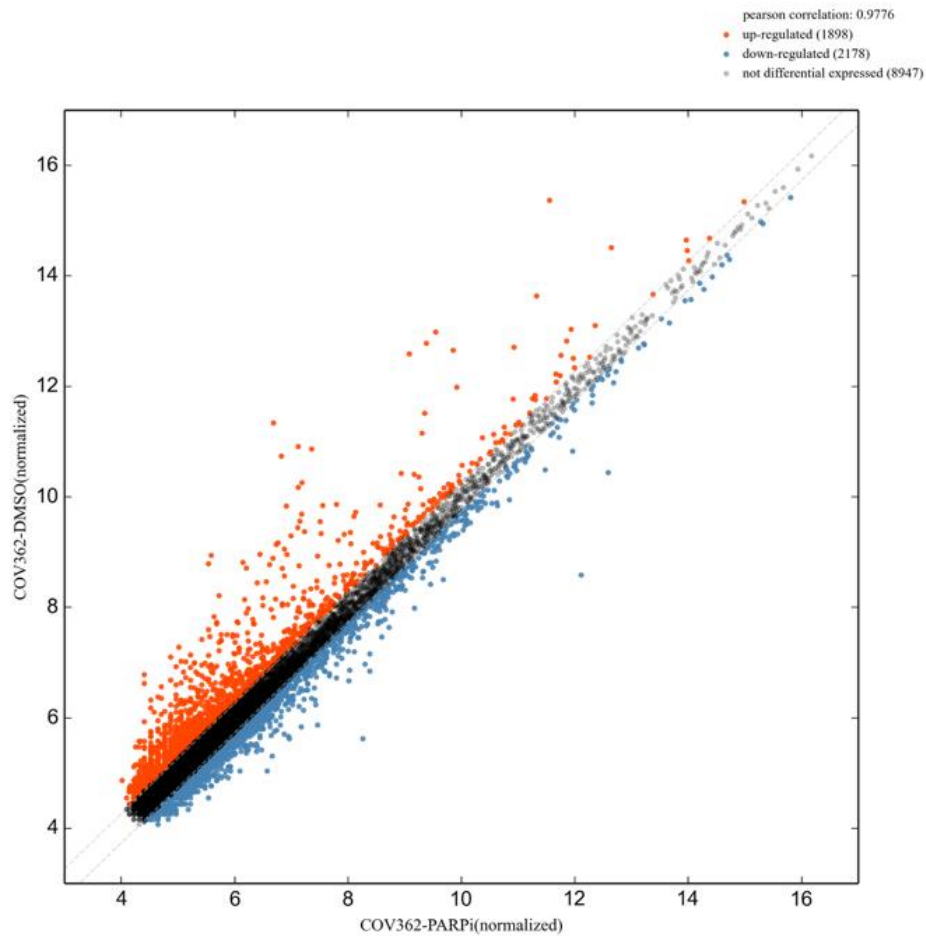


Figure 8-4 Scatter plot of circRNA expression in PARPi-resistant vs. UT

Each point represents an individual circRNA, plotted according to relative expression in the two conditions. Data shown represent the mean expression values from three biological replicates ($n = 3$). The distribution indicates a high overall correlation between parental and PARPi-resistant states, consistent with limited but distinct transcriptional reprogramming associated with PARPi exposure.

8.3.2 Chromosomal Distribution of Resistance-Linked circRNAs

The genomic distribution of host genes giving rise to differentially expressed circRNAs in resistant COV362 cells was mapped across chromosomes to explore whether changes in circRNA expression were confined to specific genomic regions or distributed more broadly. In the chemotherapy-resistant model, differentially expressed circRNAs were detected across all chromosomes, with a particularly high density on gene-rich regions such as chromosomes 1, 2, and 17 (**Figure 8-5**). This widespread distribution suggests that chemotherapy resistance involves global transcriptional reprogramming rather than the activation of discrete genomic hotspots. In contrast, the PARPi-resistant COV362 model demonstrated a broadly similar genomic pattern, with circRNAs mapped across all chromosomes and enrichment noted on chromosomes 1, 2, 17, and 19 (**Figure 8-6**). However, the overall distribution appeared more balanced, consistent with a less extensive transcriptomic shift compared to the chemotherapy-resistant model.

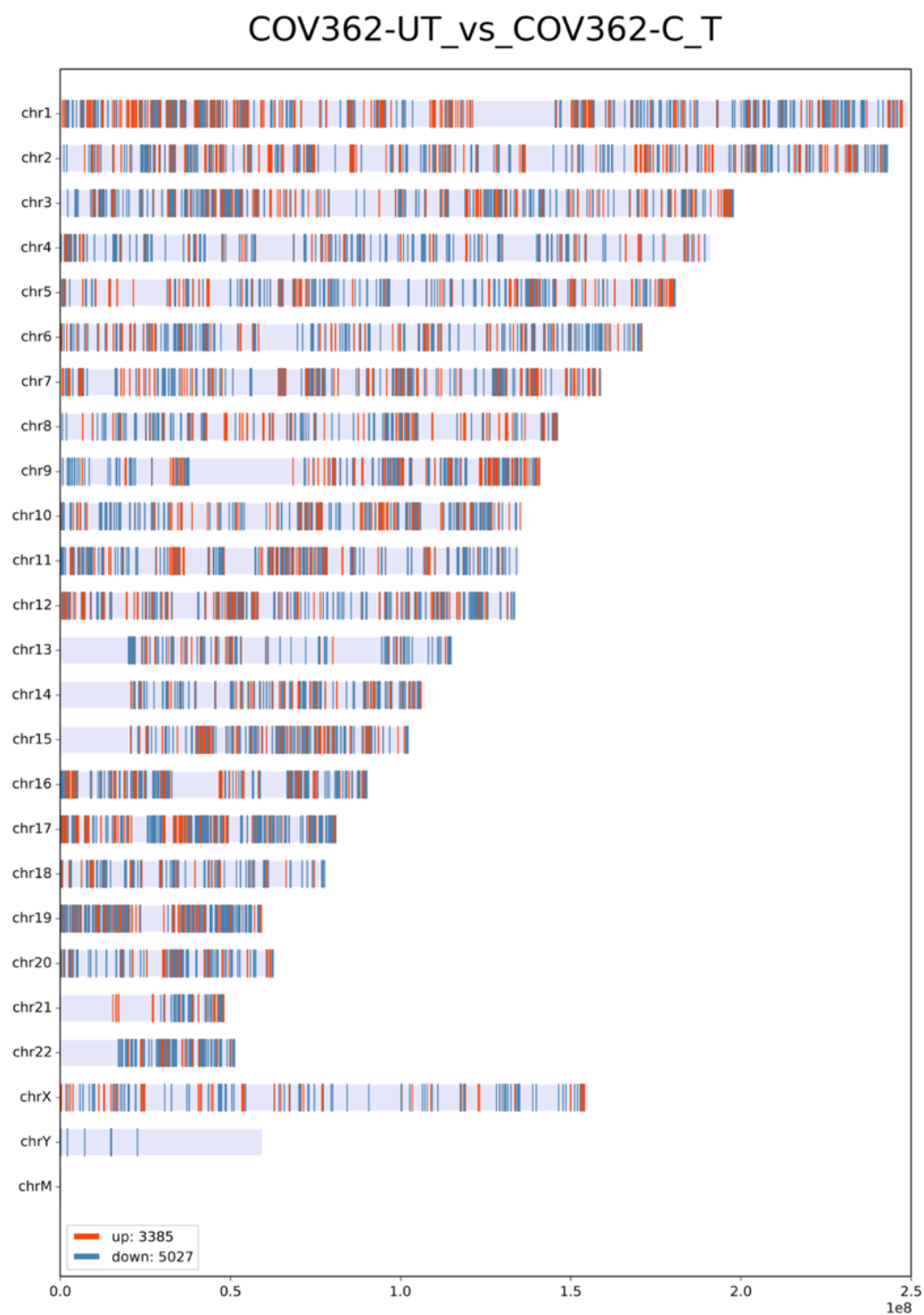


Figure 8-5 circRNA chromosomal distribution in Chemo-resistant vs. UT

Each bar represents the number of upregulated (red) and downregulated (blue) circRNAs mapped to individual chromosomes. The plot illustrates the overall genomic distribution pattern of circRNA changes between conditions.

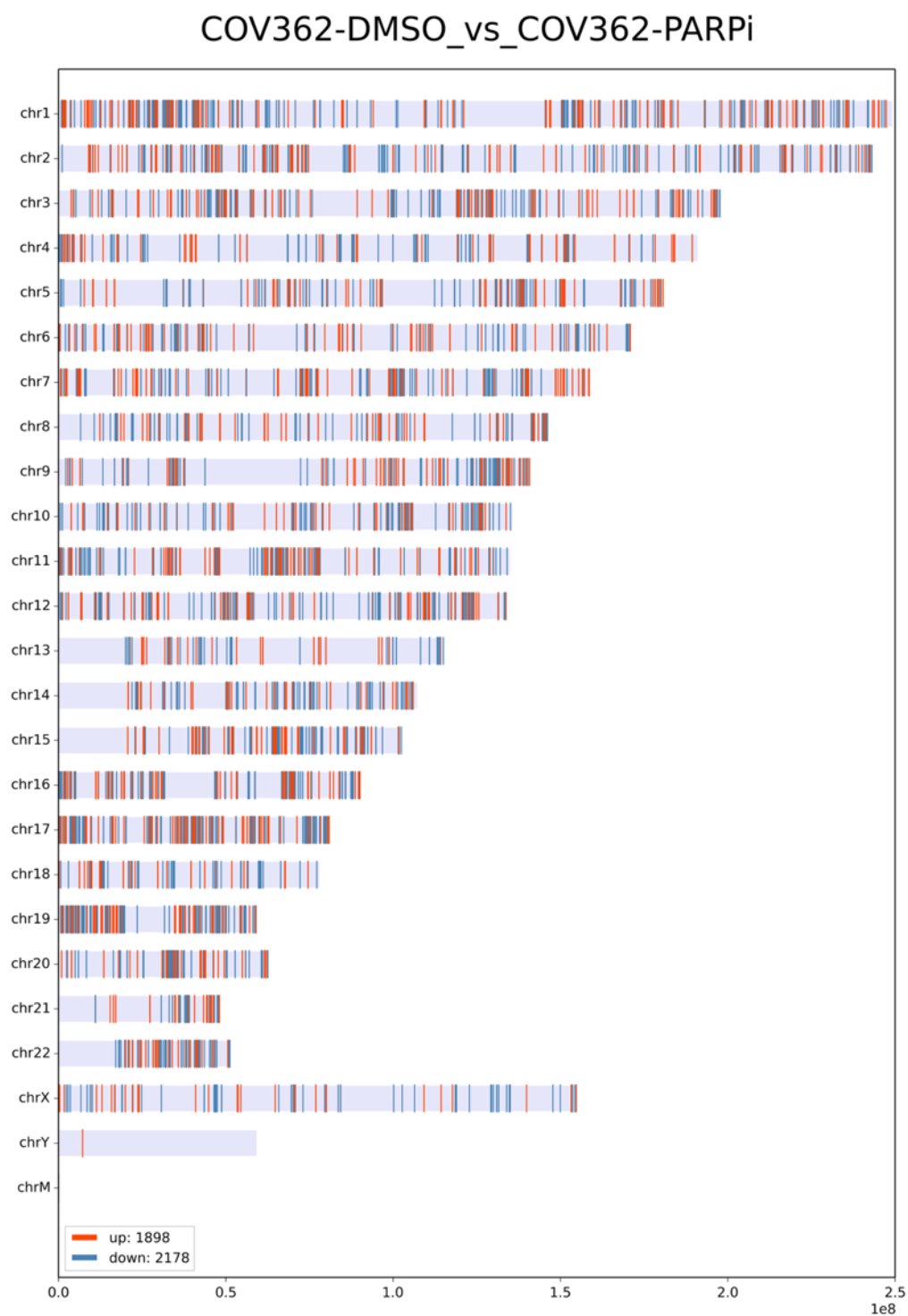


Figure 8-6 circRNA chromosomal distribution in PARPi-resistant vs. UT

Each bar represents the number of upregulated (red) and downregulated (blue) circRNAs mapped to individual chromosomes. The chart provides a visual overview of the genomic locations of circRNA alterations associated with PARPi resistance.

8.3.3 GO Enrichment in circRNAs UT vs Chemo-resistant

Gene Ontology (GO) enrichment analysis was performed on the host genes corresponding to differentially expressed circRNAs in *COV362* cells, comparing untreated and chemotherapy-resistant conditions. Host genes were identified using the Arraystar circRNA annotation file, which links each circRNA to its parental transcript based on genomic back-splice coordinates. The resulting gene list was used as input for enrichment analysis across the three GO domains: Molecular Function, Cellular Component, and Biological Process.

Significant enrichment was observed across all domains (**Figure 8-7**). Molecular Function terms predominated, particularly those associated with protein and ion binding, nucleotide binding, and catalytic activity, indicating altered interaction and enzymatic potential in the resistant state. Cellular Component terms highlighted enrichment in organelle-associated and intracellular lumen structures, while Biological Process terms were mainly related to chromatin organisation and protein modification. Collectively, these findings suggest that chemotherapy resistance in *COV362* involves broad modulation of molecular binding functions and intracellular architecture rather than isolated pathway-specific effects.

8.3.3.1 Biological Processes (BPs)

Enrichment analysis was performed on host genes derived from the differentially expressed circRNAs identified in COV362 cells, comparing untreated and chemotherapy-resistant conditions. Gene Ontology analysis revealed enrichment of biological processes associated with intracellular organisation and macromolecular modification (**Figure 8-8**). The most significantly enriched terms included cellular component organisation or biogenesis, protein and macromolecule modification, intracellular signal transduction, and chromatin remodelling. These findings suggest that chemotherapy resistance is may be accompanied by reorganisation of intracellular architecture and modulation of regulatory pathways.

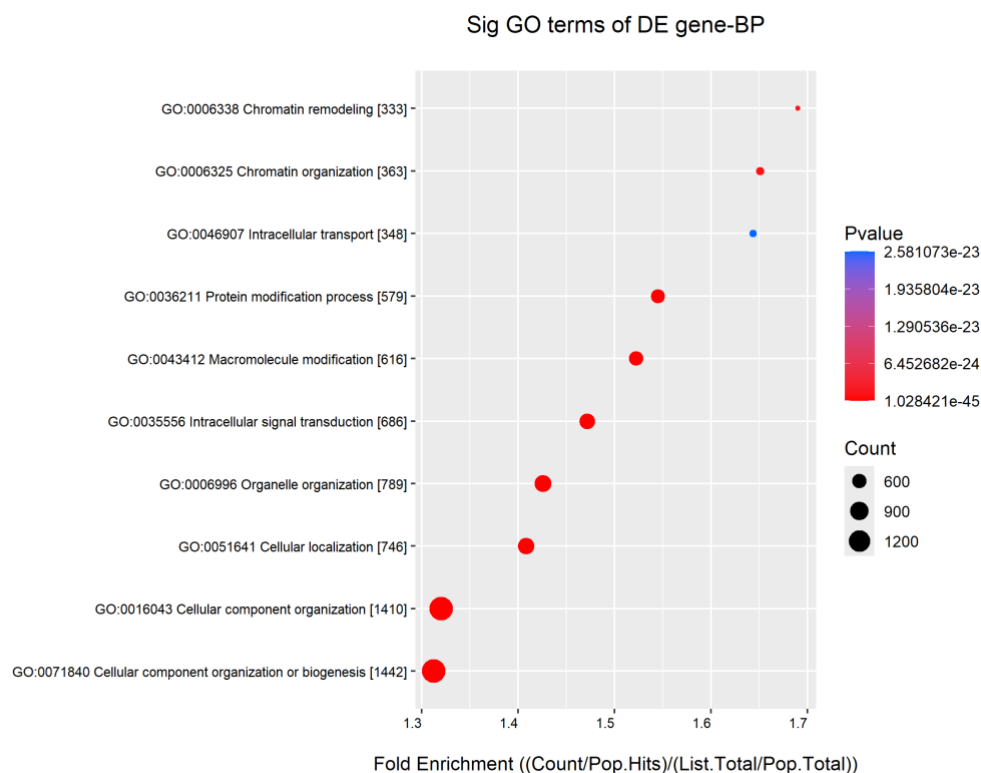


Figure 8-8 GO BP enrichment for circRNA host genes in chemo-resistant vs. UT

Dot plot showing the top significantly enriched GO Biological Process terms, ordered by fold enrichment in COV362 model. Point size reflects gene count per term, and colour represents adjusted *p*-value. Enriched terms include chromatin remodelling, intracellular transport, and protein modification, suggesting structural and regulatory reprogramming in chemo-resistant cells.

8.3.3.2 Cellular Components (CCs)

GO cellular component analysis of host genes for differentially expressed circRNAs in COV362 (untreated vs. chemo-resistant) showed enrichment in intracellular compartments, including the cytosol, nucleoplasm, and organelle lumen (**Figure 8-9**). These findings suggest widespread cellular

restructuring accompanying chemotherapy exposure, with circRNA-associated genes localised to compartments involved in protein synthesis, RNA processing, and intracellular signalling. Enrichment patterns were consistent across significant terms, reflecting large gene representation within these components (Figure 8-10).

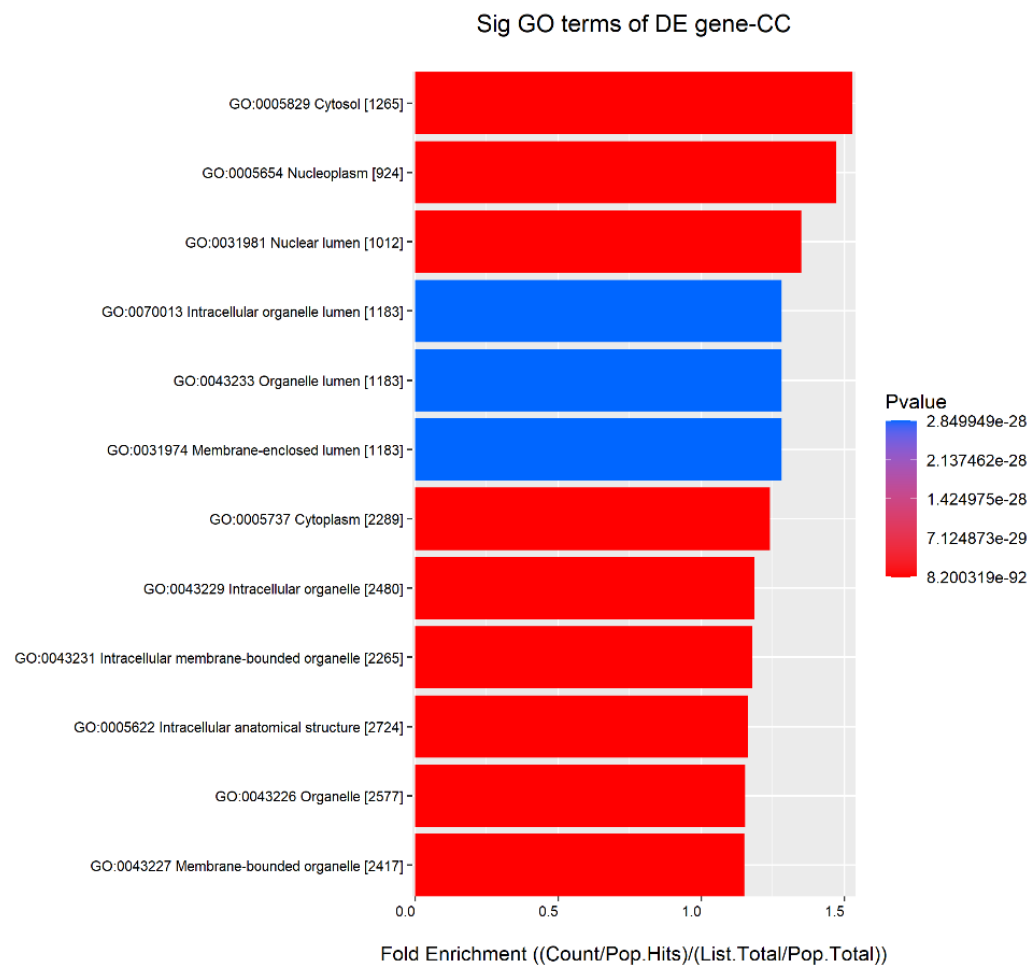


Figure 8-9 GO CC enrichment (fold enrichment) in chemo-resistant vs. UT

Bar plot showing fold enrichment for significant GO CC terms in COV362 model. Colour denotes adjusted *p*-value. Enrichment is concentrated in cytosolic and nucleoplasmic regions, consistent with intracellular reorganisation in resistant cells.

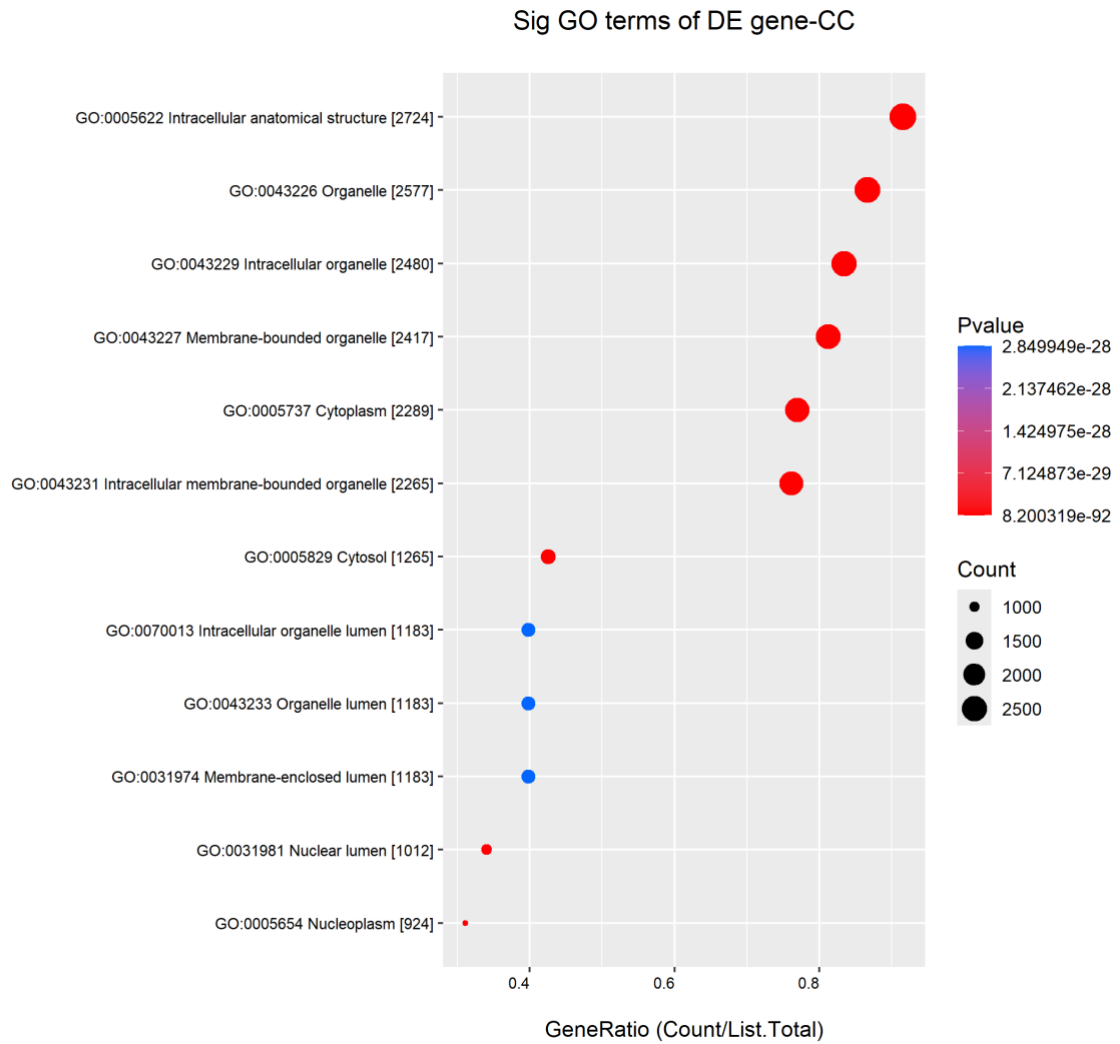


Figure 8-10 GO CC enrichment (GeneRatio) in chemo-resistant vs. UT

Dot plot showing the proportion of circRNA host genes mapping to each significant cellular component term in COV362 model. Colour represents adjusted p -value, and point size indicates gene count. Enrichment clusters were observed around cytoplasmic and organelle-associated compartments.

8.3.3.3 Molecular Function (MFs)

GO molecular function analysis of host genes for differentially expressed circRNAs in COV362 (chemo-resistant vs. UT) showed enrichment for binding and catalytic activities (**Figure 8-11**). The most significantly enriched terms included adenylyl ribonucleotide binding, ATP binding, and purine nucleotide binding, along with catalytic activity acting on proteins and ion-binding functions. These results indicate that chemotherapy resistance is associated with modulation of nucleotide and protein interaction pathways, reflecting altered energy metabolism and enzyme activity within resistant cells. Enrichment patterns were broadly consistent across terms, with comparable GeneRatio values (**Figure 8-12**).

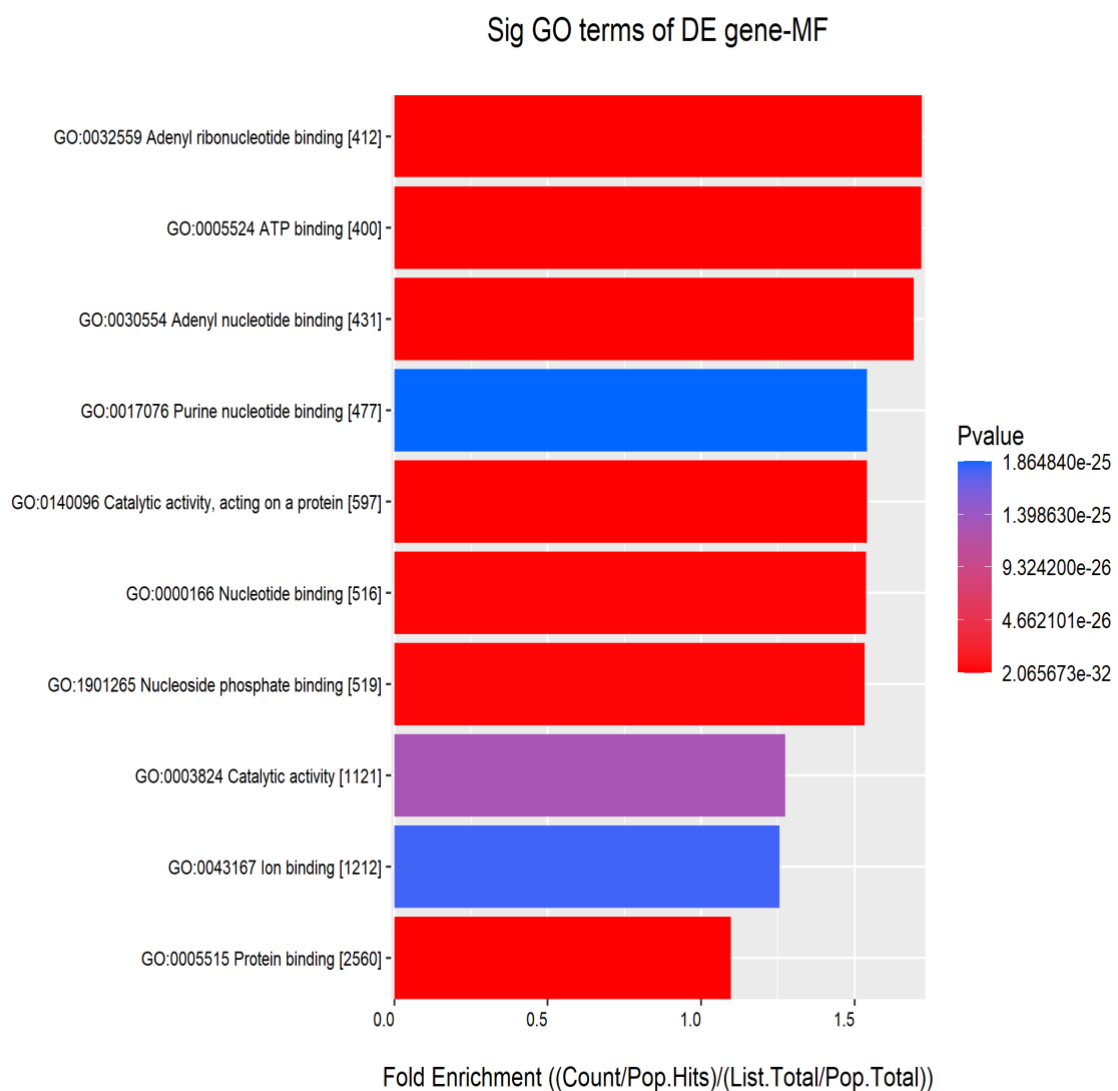


Figure 8-11 GO MF enrichment (fold enrichment) in chemo-resistant vs. UT

Bar plot showing fold enrichment for significant GO MF terms in COV362 model. Colour denotes adjusted *p*-value. Enrichment is concentrated in nucleotide- and catalytic activity categories.

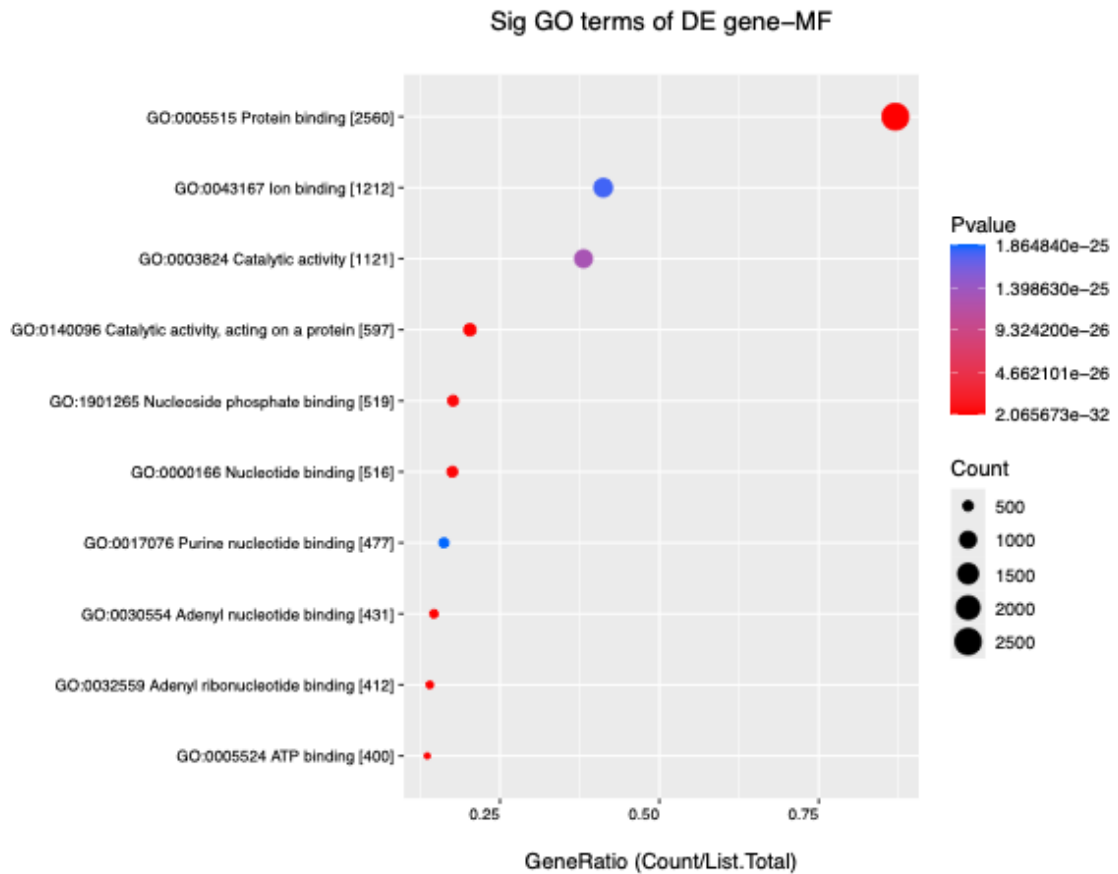


Figure 8-12 GO MF enrichment (GeneRatio) in chemo-resistant vs. UT

Dot plot showing the proportion of circRNA host genes mapping to each significant molecular function term in COV362 model. Colour represents adjusted *p*-value, and point size indicates gene count.

8.3.4 GO Enrichment in circRNAs UT vs PARPi-resistant

Significant GO enrichment was observed for host genes of differentially expressed circRNAs in COV362 cells comparing untreated with PARPi-resistant cells. Signals were detected across all three domains, with cellular component terms showing the greatest fold enrichment (**Figure 8-13**). These terms predominantly mapped to RNA-handling compartments such as ribonucleoprotein granules and nuclear or cytoplasmic bodies. Biological process and molecular function terms were fewer and of lower magnitude, indicating more focused changes in subcellular localisation rather than broad transcriptional pathway activation. Overall, the pattern supports a model in which PARPi resistance is associated with altered post-transcriptional rather than widespread disruption of core cellular processes.

8.3.4.1 Biological Processes (BPs)

Enrichment analysis for differentially expressed circRNA host genes in COV362 (untreated vs. PARPi-resistant) was dominated by cell cycle-related and mitotic control pathways (**Figure 8-14**). Top enriched terms included mitotic cell cycle, chromosome organisation, chromatin remodelling, and intracellular transport, along with localisation and organelle organisation processes. These findings suggest that PARPi resistance involves disruption of mitotic checkpoints and altered chromatin architecture, consistent with adaptive cell cycle deregulation rather than changes in RNA turnover. Fold enrichment values were moderate, reflecting coordinated but focused shifts in gene regulation.

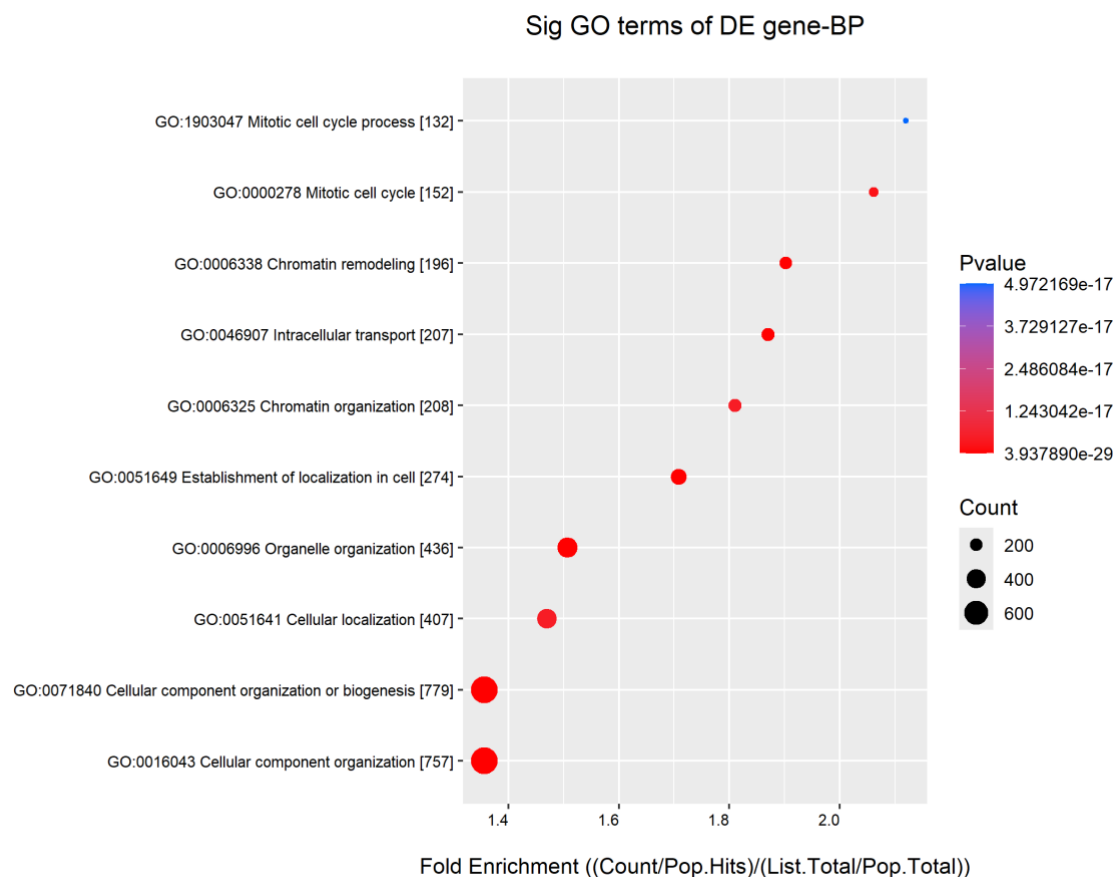


Figure 8-14 GO BP enrichment in PARPi-resistant vs. UT

Dot plot showing significantly enriched GO BP terms in COV362 model. The x-axis represents fold enrichment, colour denotes adjusted *p*-value, and point size corresponds to gene count. Enrichment highlights mitotic and chromatin organisation pathways consistent with PARPi-induced cell cycle adaptation.

8.3.4.2 Cellular Components (CCs)

GO cellular component analysis for host genes of differentially expressed circRNAs in COV362 (untreated vs. PARPi-resistant) revealed enrichment in nuclear and organelle lumen compartments

(Figure 8-15). The most significant terms included nucleoplasm, nuclear lumen, and cytosol, together with intracellular organelle and membrane-bounded organelle structures. These findings indicate that PARPi resistance is associated with reprogramming of nuclear organisation and subcellular architecture rather than RNA granule localisation. GeneRatio analysis showed that enrichment was driven by widespread localisation within intracellular and membrane-enclosed organelles **(Figure 8-16)**. Together with biological process data, these results support a model in which PARPi resistance involves altered nuclear and organelle organisation, reflecting changes in cell cycle control and protein turnover mechanisms.

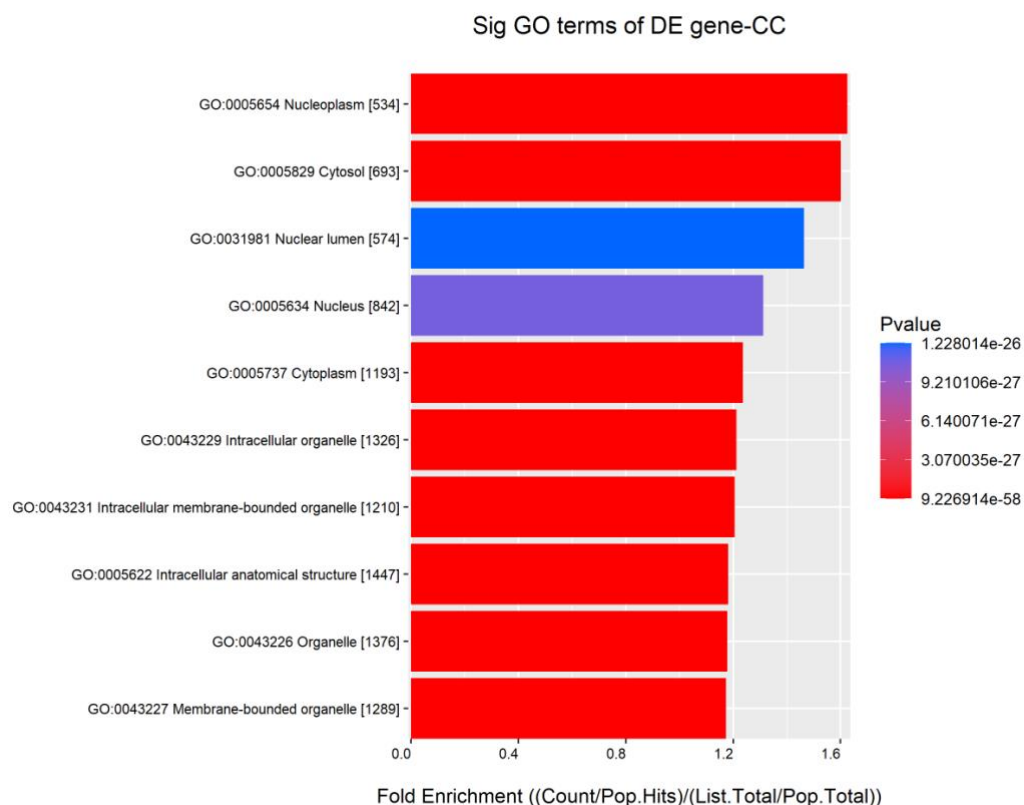


Figure 8-15 GO CC enrichment (fold enrichment) in PARPi-resistant vs. UT

Bar plot showing fold enrichment for significant GO CC terms in COV362 model. Colour denotes adjusted p -value. Enrichment is concentrated in nucleoplasmic and intracellular organelle compartments.

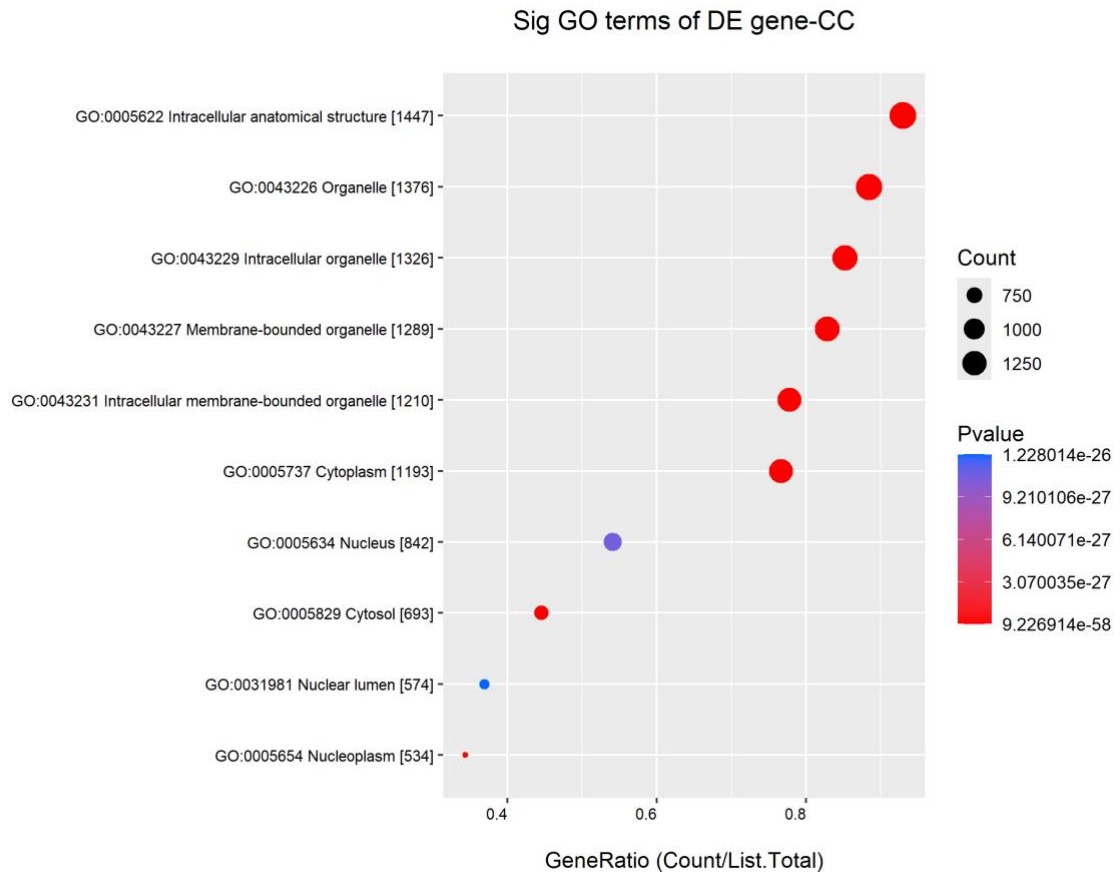


Figure 8-16 GO CC enrichment (GeneRatio) in PARPi-resistant vs. UT

Dot plot showing the proportion of circRNA host genes mapping to each significant cellular component term (GeneRatio) in COV362 model. Colour represents adjusted p -value, and point size indicates gene count. Enrichment localises to nuclear and organelle lumen structures.

8.3.4.3 Molecular Function (MFs)

GO molecular function analysis for host genes of differentially expressed circRNAs in COV362 (untreated vs. PARPi-resistant) demonstrated enrichment for binding and catalytic activities (**Figure 8-17**). The most significantly enriched terms included ATP binding, adenyly nucleotide binding, ribonucleotide binding, and purine ribonucleotide triphosphate binding, together with catalytic and protein-binding functions. These results indicate widespread modulation of nucleotide- and protein-binding capacities in the PARPi-resistant state, consistent with altered protein–protein and protein–nucleotide interaction dynamics. GeneRatio analysis showed that the largest gene proportions mapped to broad binding categories, reflecting coordinated changes across multiple functional classes (**Figure 8-18**). Collectively, the findings suggest that PARPi resistance is associated with reprogramming of protein interaction networks and signalling processes rather than redox regulation.

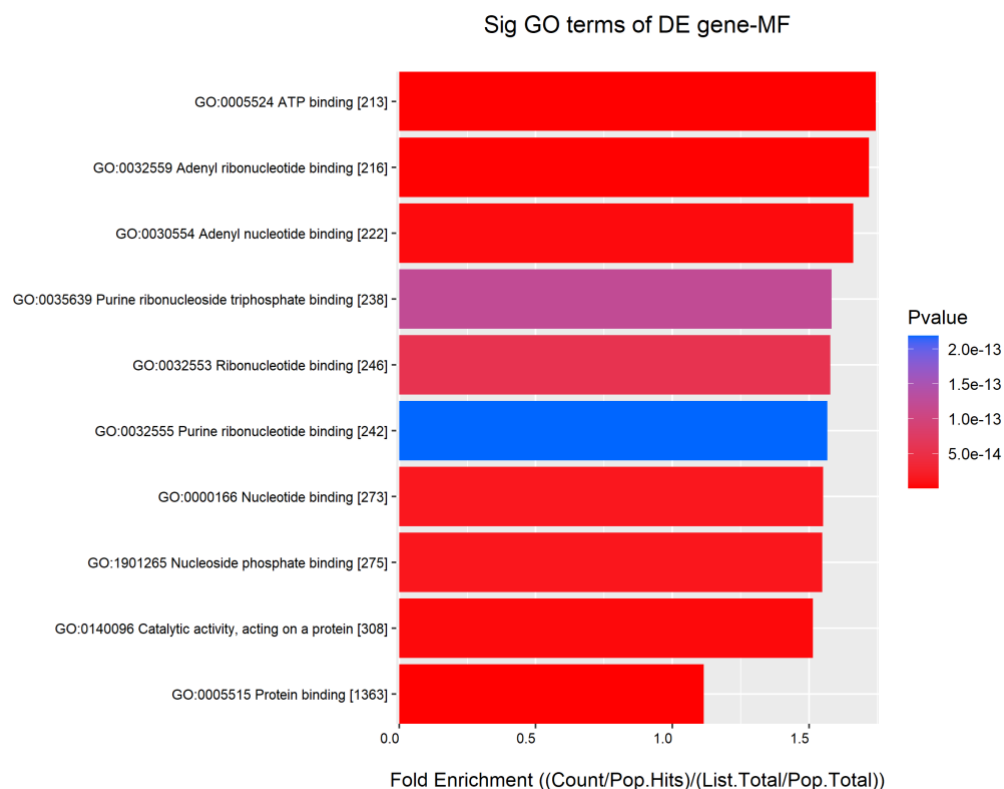


Figure 8-17 GO MF enrichment (fold enrichment) in PARPi-resistant vs. UT

Bar plot showing fold enrichment for significant GO MF terms in COV362 model. Colour denotes adjusted *p*-value. Enrichment is concentrated in ATP, nucleotide, and protein-binding categories.

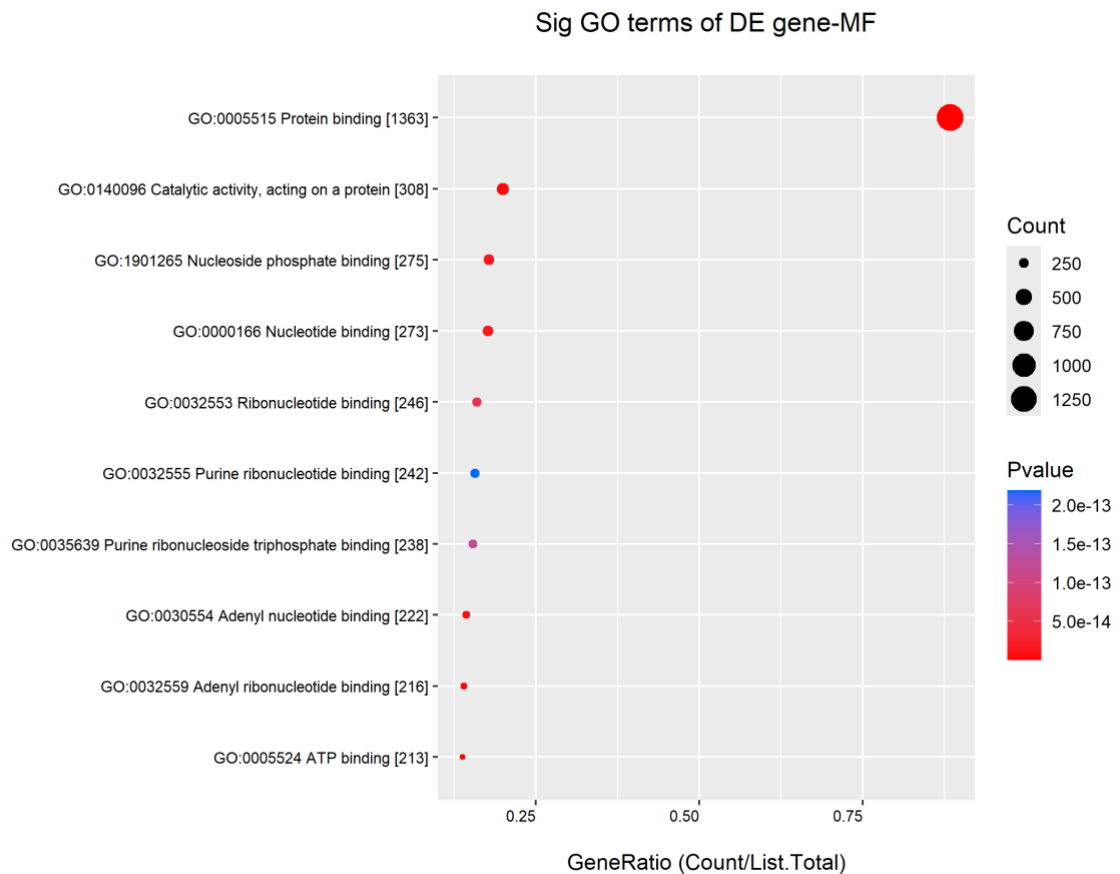


Figure 8-18 GO MF enrichment (GeneRatio) in PARPi-resistant vs. UT

Dot plot showing the proportion of circRNA host genes mapping to each significant molecular function term (GeneRatio) in COV362 model. Colour represents adjusted p -value and point size indicates gene count. Enrichment reflects predominant nucleotide- and protein-binding activities.

8.3.5 Pathways Enriched in treatment-resistant cells

To explore the biological relevance of differential circRNA expression, KEGG pathway enrichment analysis was performed using the host genes of upregulated circRNAs identified in the pairwise comparisons (chemo-resistant vs. UT; PARPi-resistant vs. UT). Mapping these genes to KEGG pathways provided a broader view of the cellular pathways associated with resistance. Consistent with the GO analyses, the focus was placed on the host genes of upregulated circRNAs because of their enhanced stability and potential functional importance.

8.3.5.1 KEGG Pathway Enrichment: COV362 Untreated vs Chemo-Resistant

Pathway analysis of upregulated circRNA host genes in chemo-resistant COV362 cells revealed enrichment for multiple oncogenic and signalling-related pathways. The most significantly enriched categories included the EGFR tyrosine kinase inhibitor resistance pathway, ErbB signalling, PI3K-Akt signalling, and other growth factor receptor-related pathways (**Figure 8-19**). Additional enriched terms encompassed neurotrophin signalling, Hippo signalling, and Ras signalling, which are frequently associated with cellular survival and stress adaptation mechanisms (4). These pathways collectively point toward activation of pro-survival and receptor tyrosine kinase signalling networks characteristic of acquired chemotherapy resistance.

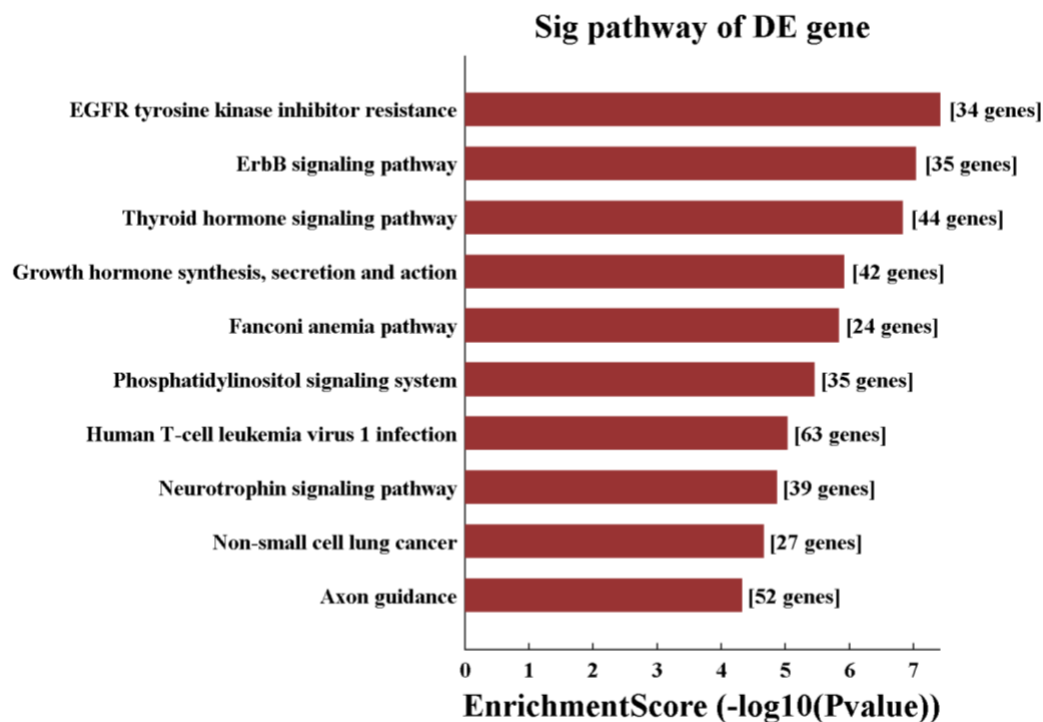


Figure 8-19 KEGG enrichment of upregulated circRNA host genes, chemo-resistant vs. UT

Bars show enrichment scores ($-\log_{10}$ adjusted p value) in COV362 model. Enriched pathways include EGFR tyrosine kinase inhibitor resistance, ErbB signalling, thyroid hormone signalling, and

neurotrophin signalling, reflecting activation of oncogenic and survival-related networks.

8.3.5.2 8.3.1.10 KEGG Pathway Enrichment: COV362 Untreated vs PARPi-Resistant

In the PARPi-resistant comparison, upregulated circRNA host genes were predominantly mapped to pathways involved in cell-cycle regulation, DNA damage response, and signal transduction (**Figure 8-20**). Enriched pathways included EGFR signalling, ErbB signalling, PI3K-Akt signalling, Hippo signalling, neurotrophin signalling, and pathways in cancer. Several of these pathways overlap with those observed in the chemo-resistant state, reflecting engagement of shared oncogenic and stress response circuits. The prominence of cell-cycle and DNA repair–related pathways suggest a potential role for circRNA-mediated modulation of replication stress and checkpoint regulation in the development of PARPi resistance

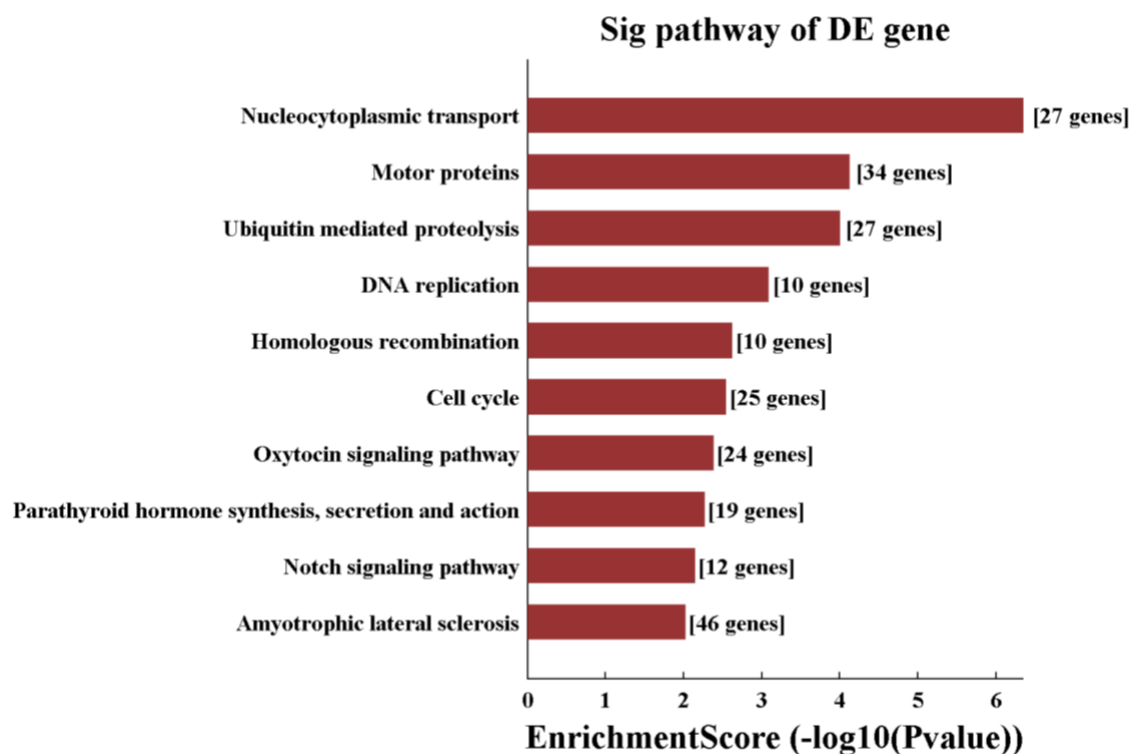


Figure 8-20 KEGG enrichment of upregulated circRNA, PARPi-resistant vs. UT

Bars show enrichment scores ($-\log_{10}$ adjusted p value) in COV362 model. Enriched pathways include nucleocytoplasmic transport, motor protein activity, ubiquitin-mediated proteolysis, DNA replication, and cell-cycle regulation, indicating altered replication dynamics and proteostasis in the PARPi-resistant state.

8.3.6 Differential circRNA Profiles in Resistant Vs. Parental OC Cell Lines

Differential-expression analysis was performed for the two pairwise comparisons: untreated vs.

chemo-resistant and DMSO-treated vs. PARPi-resistant COV362 cells. circRNAs showing increased expression relative to their respective parental controls were identified using a fold-change cut-off of ≥ 1.2 and $p \leq 0.05$. The resulting datasets captured the broader repertoire of circRNAs associated with treatment resistance. From each comparison, the three circRNAs with the highest fold-change were selected for subsequent characterisation and validation. Ranking events by absolute fold change highlighted distinct candidates in each model. In the chemo-resistant comparison (**Table 6-3**), top circRNAs arose from SENP3-EIF4A1 (hsa_circ_0007008), TSNARE1 (hsa_circ_0085803), and SLC7A5 (hsa_circ_0040800). In the PARPi-resistant comparison (**Table 8-2**), prominent circRNAs mapped to ATP5SL (hsa_circ_0051239; hsa_circ_0051238) and ZNF652 (hsa_circ_0003258). These ranked lists informed selection of candidates for validation and support partially shared but overall distinct circRNA programmes in chemo- and PARPi-resistant states.

circRNA	FC (abs)	Annotations			
		Alias	source	chrom	GeneSymbol
hsa_circRNA_007008	214.32902	hsa_circ_0007008	circBase	chr17	SENP3-EIF4A1
hsa_circRNA_104718	4.54649	hsa_circ_0085803	circBase	chr8	TSNARE1
hsa_circRNA_040800	3.6237513	hsa_circ_0040800	circBase	chr16	SLC7A5

Table 8-1 Top three upregulated circRNAs in chemo-resistant vs untreated

Differential expression was determined using a fold-change (FC) cut-off of ≥ 1.2 . The top three circRNAs ranked by absolute fold change (FC abs), with corresponding circBase aliases, chromosomal origin (chrom), and host gene symbols.

circRNA	FC (abs)	Annotations			
		Alias	source	chrom	GeneSymbol
hsa_circRNA_051239	11.5206758	hsa_circ_0051239	circBase	chr19	ATP5SL
hsa_circRNA_102116	6.230028	hsa_circ_0003258	circBase	chr17	ZNF652
hsa_circRNA_051238	4.4302144	hsa_circ_0051238	circBase	chr19	ATP5SL

Table 8-2 Top three upregulated circRNAs in PARPi-resistant vs untreated

Differential expression was determined using a fold-change (FC) cut-off of ≥ 1.2 . The top three circRNAs ranked by absolute fold change (FC abs), with corresponding circBase aliases, chromosomal origin (chrom), and host gene symbols.

8.3.7 Combined Resistance Overlap

For the overlap analysis, circRNAs were considered differentially expressed if they met a fold-change threshold of ≥ 2 and $p \leq 0.05$. Applying these criteria identified 1,120 circRNAs that were upregulated in both chemotherapy- and PARPi-resistant COV362 sublines (**Figure 8-21**).

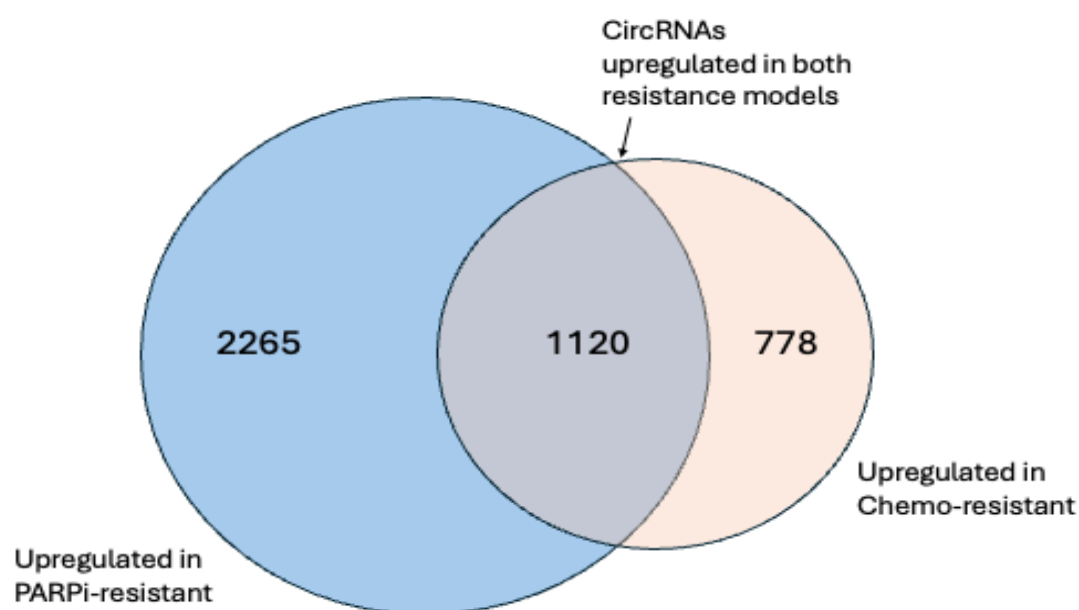


Figure 8-22 Venn diagram showing circRNAs upregulated in resistant vs. UT cells.

The intersection represents circRNAs upregulated in both chemo-resistant and PARPi-resistant states. The diagram was generated using Bioinform Venn Diagram Tool (491) and refined in Microsoft PowerPoint (Version 16.101.3) for visual clarity.

8.3.8 Validation of circRNA Expression in Resistant Cell Lines

8.3.8.1 Selection, Design, and Optimization of circRNAs

Divergent primer pairs were custom designed to span the back-splice junction of the circRNAs listed in **Table 8-1** and **Table 8-2**; one primer overlapped the junction by at least 8 nucleotides to prevent amplification of linear transcripts (**Table 8-3**). Amplicons were targeted at 90–150 bp and an optimal primer T_m of ~58 °C. Primers were screened by endpoint PCR and four of the six primer sets were validated (**Figure 8-23**, **Figure 8-24**) (two from the chemo-resistant set and two from the PARPi-resistant set) and were advanced to qPCR.

No.	circRNA (Alias)	Forward Primer	Reverse Primer
1	hsa_circ_0007008	5°- TCC TAG CAC CCA C T GAG AA -3'	5'- GGC CTG GGT GGC TTT TAG AT -3'
2	hsa_circ_0085803	5'- CTC CAG TGT GAC CTC CTT GG -3'	5°- GGC GAT GGATCC GTA TGA CA -3'
3	hsa_circ_0040800	5'- GCT AAC CTG TTA CAC TGGGCT -3'	5-' TCACGTGTAGCAGTTCACG -3'
4	hsa_circ_0051239	5-' ACA CGC TGT CCT TAC ACT GG -3'	5-' TCCCACATCCTGAACAATCC -3'
5	hsa_circ_0003258	5'- GCA CAA ACA GTT CAT GIG CCA -3'	5-' AAG GAG TGC ACC TTG AGT GA -3'
6	hsa_circ_0051238	5-' ACA CGC TGT CCT TAC ACT GG -3'	5-' CTT CG ATTGCC CTC TGG GG -3'

Table 8-3 Divergent primer pairs for selected circRNAs

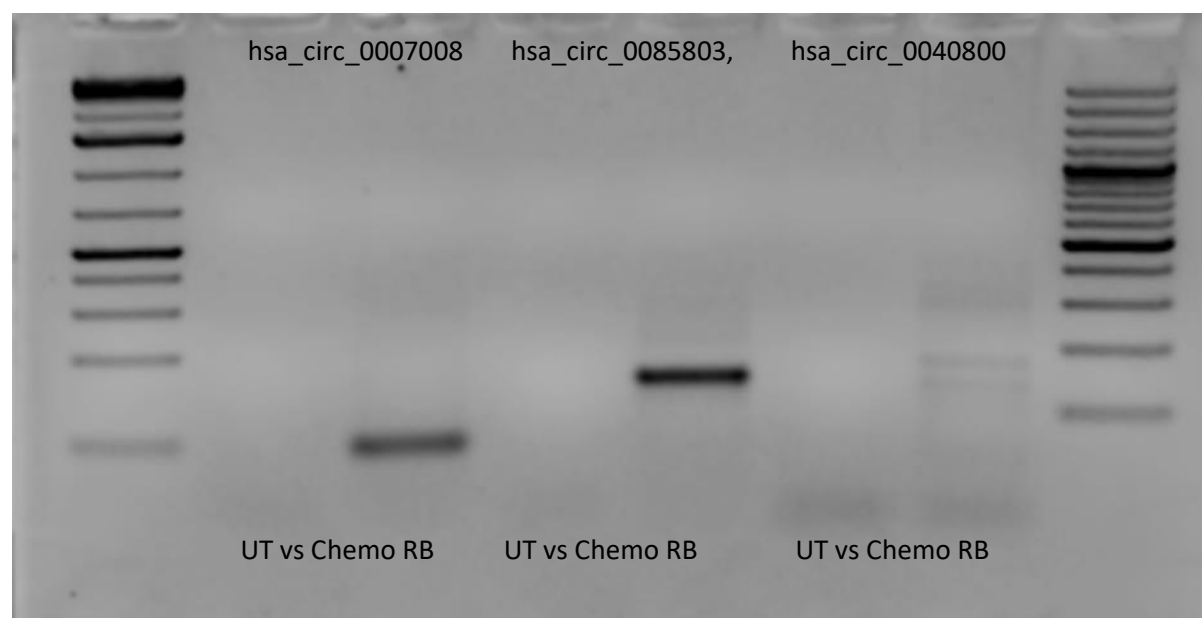


Figure 8-23 Visualisation of endpoint PCR products in (chemo-resistant COV362 model).

Left and right outer lanes: DNA ladder. Each target is shown as a lane pair with untreated (UT) on the left and chemo-resistant (Chemo RB) on the right. Targets (left-to-right pairs): hsa_circ_0007008, hsa_circ_0085803, hsa_circ_0040800.

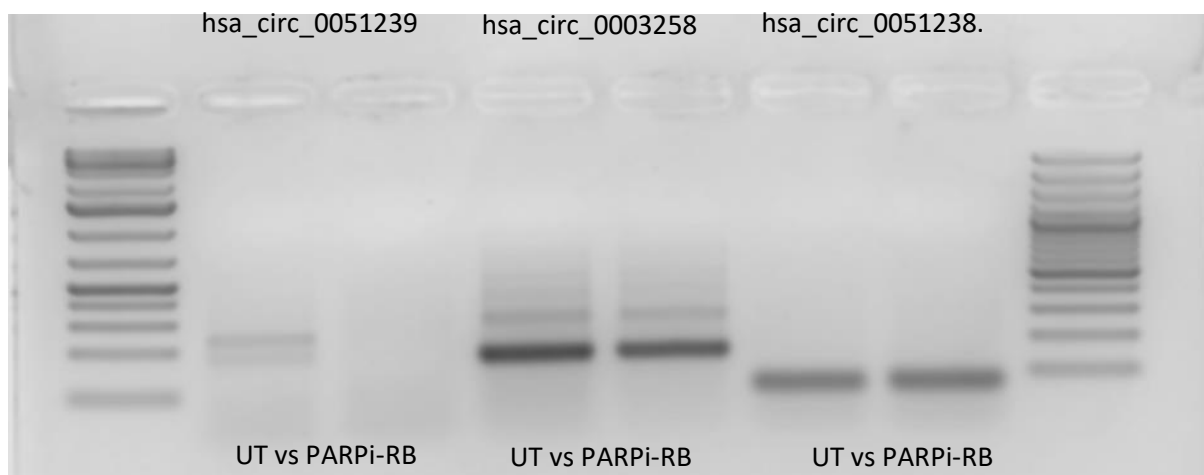


Figure 8-24 Visualisation of endpoint PCR products in , PARPi-resistant COV362 model.

Left and right outer lanes: DNA ladder. Each target is shown as a lane pair with untreated (UT) on the left and PARPi-resistant on the right (PARPi-RB). Targets (left-to-right pairs): hsa_circ_0051239, hsa_circ_0003258, hsa_circ_0051238. Amplicons span the back-splice junction; expected product sizes ~90–150 bp.

8.3.8.2 Validation of circRNA in cell lines

The four circRNA primers that were successfully optimised by endpoint PCR were validated by qPCR. in the chemo-resistant comparison, circRNA 85803 was increased relative to parental cells, whereas circRNA 7008 showed no difference (Figure 8-25). In the PARPi-resistant comparison, circRNA 3258 was increased relative to UT, while circRNA 51238 showed no discernable difference. These findings prioritised circRNA 85803 and circRNA 3258 for follow up as candidate markers of resistance.

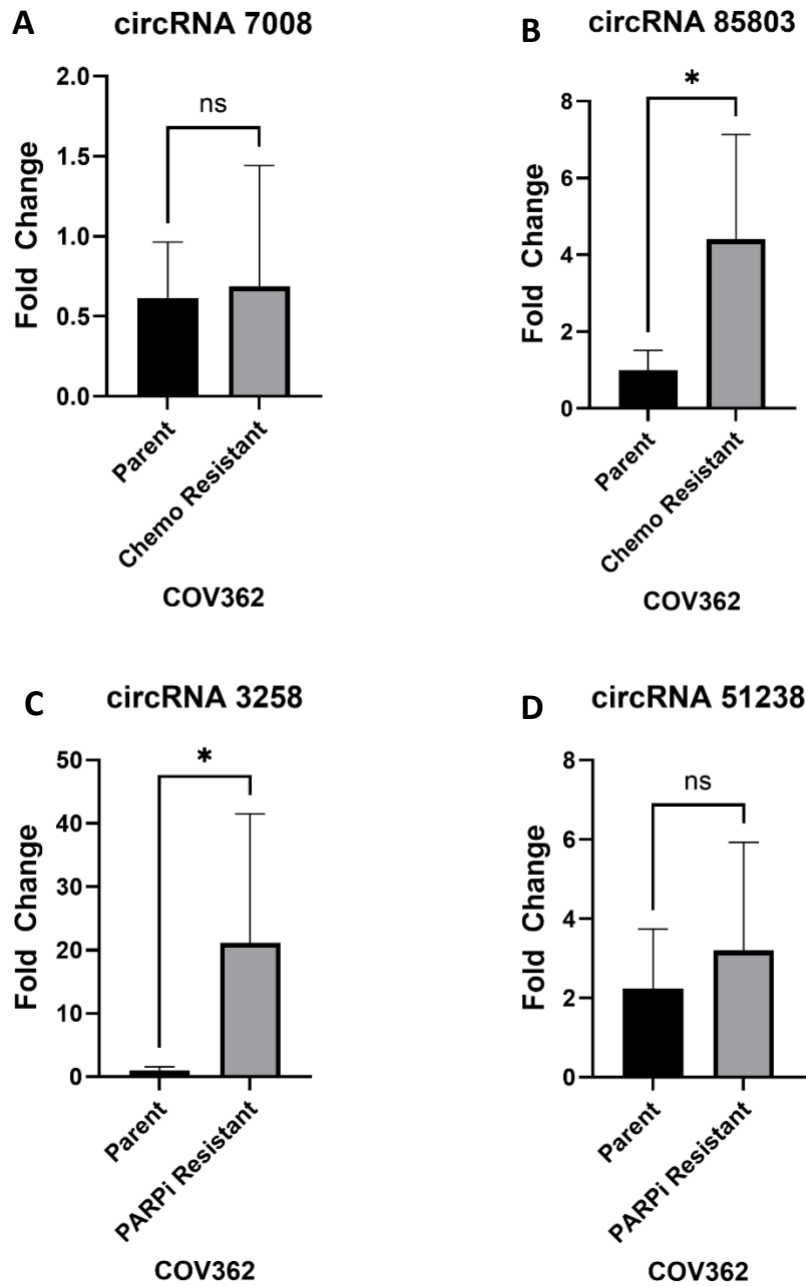


Figure 8-25 qPCR validation of circRNA expression in COV362 resistant models

(A) circRNA_7008 in parental vs. chemotherapy-resistant cells. (B) circRNA_85803 in parental vs. chemotherapy-resistant cells. (C) circRNA_3258 in parental vs. PARPi-resistant cells. (D) circRNA_51238 in parental vs. PARPi-resistant cells. Expression was normalised to the housekeeping control and presented as fold change relative to untreated (UT) parental cells. Data are graphed as mean $\pm$ SEM ($n = 3$).

8.4 Discussion

The overarching aim of this work is to generate a catalogue of circRNAs that may serve as

biomarkers of therapeutic resistance in OC. By integrating array profiling with validation in cell lines and translational samples, the findings of this chapter provide insight into the molecular signatures of resistance and lay the groundwork for future mechanistic and clinical studies. This chapter's findings suggest that acquired resistance to *BRCA1*-mutant HGSOC involves circRNA-mediated structural and regulatory adaptation, particularly within nuclear and chromatin domains. Across both chemo- and PARPi-resistant COV362 sublines, circRNA profiles distinguished resistant from UT cells. GO enrichment revealed consistent themes in structural, binding, and regulatory processes. In the chemo-resistant comparison, enriched Biological Process terms clustered around cellular organisation, chromatin remodelling, intracellular transport and localization; Cellular Component enrichment emphasised cytosol, nucleoplasm, nuclear lumen, and membrane-bound organelles; and Molecular Function terms were dominated by nucleotide/ATP binding, ion and protein binding, and catalytic activity. In the PARPi-resistant contrast, the overall pattern was similar but showed stronger nuclear emphasis, with GO categories centred more on chromatin organization, mitotic and cell cycle terms, nucleoplasmic components, and binding functions. Together, these data support a model in which circRNA changes accompany global structural and regulatory rewiring, especially in nuclear and chromatin domains, rather than discrete activation of a single canonical pathway.

Compared with the chemo-resistant model, the overall number of differentially expressed circRNAs was lower, consistent with a more modest transcriptomic shift associated with PARPi resistance. Across both resistant models, no single chromosomal locus dominated circRNA deregulation. Instead, resistance-linked circRNAs arose from multiple chromosomes, suggesting that circRNA reprogramming in COV362 reflects a genome-wide rather than locus-specific process. Further, high fold enrichment values reflected large contributing gene sets, supporting the robustness of these findings. Overall, the results suggest that chemotherapy resistance in COV362 involves coordinated alterations in chromatin structure, signalling, and protein regulation, consistent with widespread cellular adaptation. Together with biological process data, these results indicate that chemotherapy resistance is associated with broad reorganisation of intracellular architecture rather than confinement to specific RNA granule compartments.

Some of these observations align with expectations from circRNA and resistance biology in the literature, though there are also areas of divergence. It is well established that circRNAs contribute to drug resistance by acting as miRNA sponges or interacting with RNA-binding proteins, thereby modulating gene expression networks in cancer and OC in particular (169, 172, 188). Many prior reports of circRNAs in OC have emphasized roles in pathways such as PI3K/AKT, Wnt, apoptosis, and EMT, often through cytoplasmic signaling effects (e.g. via miRNA sponging)(492-496). In contrast, these findings point more strongly toward circRNAs engaging nuclear/chromatin structure,

intracellular trafficking, and binding-mediated regulatory control, rather than primarily modulating growth factor signalling alone. This shift toward structural/regulatory involvement is consistent with newer work showing that some circRNAs interact directly with chromatin remodelers or influence nuclear RNA-binding proteins (RBPs). For example, circRNAs that bind RBPs and affect chromatin occupancy or transcription (497), reflecting a nuclear-level regulatory role beyond the classic miRNA sponging model.

The differing emphases in the two resistance settings also make sense from a mechanistic perspective. For chemo-resistance, the stronger signal in intracellular transport and localization GO categories may reflect adaptation to cytotoxic damage and drug stress. For example, circRNAs may help reroute RNAs or proteins, stabilize stress granules or organellar responses, or buffer damage by localization-based sequestration (170, 498, 499). For PARPi resistance, the more nuclear-focused GO terms could reflect the necessity for resistant cells to re-establish genome stability, rewire chromatin access, or adjust mitotic checkpoints. This is plausible given that one major route to PARPi resistance is restoration of homologous recombination or bypass repair compensatory pathways (500, 501), which inherently involve nuclear architecture and chromatin repair machinery.

The identification of *hsa_circ_0085803* (*circRNA_104718*) as one of the most upregulated circRNAs in the chemo-resistant dataset provides a concrete example supporting this model. In published studies, *circRNA_104718* is overexpressed in glioma and hepatocellular carcinoma, where it regulates *miR-218-5p* and downstream targets such as *HMGB1* and *TXNDC5*, thereby promoting proliferation, invasion and suppression of apoptosis (502, 503). Its upregulation in the chemo-resistant HGSOC model suggests that a similar *miR-218-5p*-related regulatory axis may be active in OC, contributing to resistance through chromatin repair or stress-response pathways. This observation supports the idea that circRNAs can modulate conserved post-transcriptional networks across tissue types, potentially repurposing them to promote chemoresistance in this context.

However, there are caveats and points of departure worth noting. Some enriched GO terms are supported by only a few genes; such low counts inflate fold-enrichment and increase false-positive risk, so these terms are interpreted cautiously. Moreover, many earlier circRNA-resistance studies have used less representative OC cell lines (e.g. A2780 or SK-OV-3)(184, 204, 504-509) or focused solely on cisplatin resistance models (254), which may not capture the chromatin or DNA-repair pressures characteristic of a *BRCA1*-mutant, PARPi-exposed background. Consequently, the observed circRNA-linked chromatin and nuclear shifts in this study may be novel, as, no previous analyses have examined matched chemo- and PARPi-resistant models in a true HGSOC/HRD setting. Nonetheless, emerging evidence supports the plausibility of these findings, with recent studies showing that certain circRNAs regulate chromatin-associated RNA-binding proteins (RBPs) or

influence transcription through modulation of R-loop dynamics (510).

In summary, this chapter's findings suggest that acquired resistance in *BRCA1*-mutant HGSOC involves circRNA-mediated structural and regulatory adaptation, particularly in nuclear and chromatin domains. The overlap in broad GO themes across resistance modes suggests a shared underlying framework (e.g. maintenance of genome integrity, binding interactions, intracellular logistics), overlaid by therapy-specific refinements (e.g. nuclear remodelling under PARPi, transport adaptations under chemo). The identification of circRNA_104718 as a prime candidate in the x model links these global patterns to a candidate with mechanistic precedent in cancer. While additional models and further validation is needed, this integrated view positions circRNAs not just as passive markers but as active drivers of resistance adaptation, opening new avenues for mechanistic and biomarker research in HGSOC.

Discussion, Future Works and Conclusion

Chapter Nine

9 Discussion, Future Works and Conclusion

9.1 Discussion

OC continues to represent one of the most lethal malignancies among women worldwide, largely due to late diagnosis, high recurrence rates, and the eventual emergence of resistance to platinum-based chemotherapy and PARPi. Despite substantial advances in understanding the molecular underpinnings of OC - particularly the role of *BRCA1* and *BRCA2* in homologous recombination repair (HRR) - clinical translation of these insights remains incomplete. The growing complexity of genomic data, fragmented health information systems, and limited access to harmonised biobanks have all constrained the ability to integrate molecular findings into clinical care.

This thesis was designed to address these gaps through a multidimensional approach encompassing epidemiologic mapping, registry infrastructure development, and translational molecular biology. Across six results chapters, the work has combined clinical observation, data integration, *in silico* profiling, and *in vitro* experimentation to generate both scientific insights and tangible research infrastructure. Taken together, these components form a coherent body of work that not only elucidates biological mechanisms of treatment resistance and *BRCA*-associated disease but also establishes the foundations for future collaborative, patient-centred research in Ireland

The research presented in this thesis was designed to address these challenges across complementary translational, clinical and infrastructural domains. Collectively, these studies bridge the spectrum from molecular discovery to real-world implementation, establishing the foundations required for future large-scale patient-centred research within the Irish context.

At the epidemiologic level, nationwide analysis of *BRCA1* and *BRCA2* germline and somatic pathogenic variants in OC (Chapter 3) defined, for the first time, the prevalence and clinical correlates of hereditary risk in Irish patients, demonstrating that approximately 20% of women harboured a clinically actionable variant (CAV). This work also underscored gaps in systematic testing, data linkage, and follow-up, highlighting the fragmented infrastructure that currently limits comprehensive national surveillance. Parallel analysis of young-onset OC revealed distinct clinico-pathological features and emphasised the unmet need for earlier genetic assessment and targeted support pathways for this population. These findings provided both the rationale and empirical grounding for the subsequent design and launch of the CAmPaiGn registry (Chapter 4).

The establishment of the Cancer-Predisposing Genetic Alterations (CAmPaiGn) pilot registry represents one of the most tangible infrastructural outputs of this thesis. This initiative addressed a key structural deficit in Irish oncology - the absence of a coordinated national platform capturing longitudinal data on patients with hereditary or genomically defined cancers. The registry's

framework was built around interoperability, data governance, and patient-centred design. Engagement with stakeholders—including clinicians, data managers, bioinformaticians, and patient advocacy groups such as BRCA Ireland and Lynch Syndrome Ireland—ensured that the design was both technically robust and ethically sound. Importantly, patient and public involvement (PPI) was embedded as a core design principle rather than a retrospective add-on. The PPI survey demonstrated that participants not only supported data sharing for research but also prioritised transparency, clinical feedback, and consent renewability—insights that now inform governance design. This participatory infrastructure offers a scalable, sustainable model for future national registries integrating genomic, clinical, and outcome data, aligning with international standards and FAIR data principles.

At the molecular level, this thesis advances understanding of *BRCA*-associated and treatment-resistant OC biology. Using a combination of *in silico* analyses and *in vitro* validation, miRNAs and circRNAs were profiled across *BRCA*-mutant (KURAMOCHI, *BRCA1*; COV362, *BRCA2*) and wildtype (OVSAHO) models, as well as chemotherapy- and PARPi-resistant derivatives (Chapters 5–8). These studies revealed distinct regulatory networks, including upregulation of *miR-652-3p* and *miR-3613* across *BRCA*-mutant lines and circRNAs linked to chromatin organisation and protein modification in resistant models. GO and KEGG enrichment analyses further mapped these transcripts to pathways involved in intracellular architecture, DNA repair, and protein processing—consistent with adaptive rewiring during acquired resistance. These findings add to emerging literature on non-coding RNA networks as modulators of *BRCA*-related phenotypes and therapeutic response.

Methodological innovation also featured strongly in this work. The development of clinically relevant chemotherapy- and PARPi-resistant OC models (Chapter 7) provided a rigorously validated resource for future mechanistic studies. The project's focus on pharmacokinetic alignment—using drug concentrations reflective of achievable plasma exposure—helped bridge a critical gap between experimental dosing and clinical relevance. Comparative viability and BrdU proliferation assays, alongside STR profiling and efflux marker assessment, established these models as stable and biologically credible tools for downstream molecular profiling. While limitations such as supraphysiological exposure and heterogeneity of resistance mechanisms were acknowledged, these models provide an invaluable preclinical scaffold for dissecting resistance phenotypes and testing targeted interventions.

In combination, the laboratory and registry components of this thesis demonstrate how research infrastructure can act as a springboard for translational advancement. The resistant models and circRNA/miRNA datasets represent the mechanistic discovery arm; the registry and linked clinical data infrastructure represent the translational delivery arm. When integrated, these enable a

seamless pipeline from bench to population-level implementation. For example, circRNAs identified here (such as hsa_circ_400223 and hsa_circ_008026) could potentially be prioritised for validation using tissue microarrays constructed from archived FFPE OC specimens. Similarly, prospective collection of blood samples for cell-free RNA and exosomal cargo, could be embedded within the CAmPaiGn consent framework, and could enable minimally invasive biomarker validation in future clinical cohorts.

The future trajectory of this research should therefore focus on systematic validation and clinical translation. Analytical validation requires reproducibility across laboratories and platforms, including the use of RNase R digestion, back-splice junction sequencing, RT-qPCR or RNA in situ hybridisation. Clinical validation will depend on access to adequately powered, independent cohorts - achievable through national and international collaboration. Collaborative partnerships with high-volume academic centres, including those with access to annotated tumour banks and longitudinal clinical data, will be essential. These collaborations could facilitate the construction of tissue microarrays spanning *BRCA*-mutant and wildtype OC, allowing multiplex assessment of circRNA and miRNA biomarkers using automated digital pathology pipelines. The registry's architecture will also allow stratification by genomic subtype, therapy exposure, and survival, enabling precision correlation of molecular signatures with clinical endpoints.

From a broader perspective, this thesis underscores that scientific discovery and infrastructural development are mutually reinforcing. The identification of candidate biomarkers is only meaningful within systems capable of validating and applying them in clinical practice. The CAmPaiGn registry, therefore, does more than catalogue patients - it represents the first step towards a national ecosystem of translational readiness. This ecosystem integrates governance, data linkage, PPI engagement, and biobanking under a unified ethical framework. By aligning discovery science with data infrastructure, Ireland can position itself to participate in and lead multicentre biomarker studies, including European reference network collaborations in hereditary and rare cancers.

In conclusion, this thesis establishes both conceptual and practical foundations for a modern, patient-centred translational research pipeline in OC. It demonstrates that sustainable progress requires parallel investment in infrastructure, inclusion, and innovation: infrastructure through national registries and biobanking frameworks; inclusion through meaningful PPI and stakeholder partnerships; and innovation through discovery of novel non-coding RNA biomarkers that can stratify treatment response. Collectively, these outputs form the scaffolding for a next generation of Irish-led translational research in hereditary and treatment-resistant ovarian cancer—one capable of moving from molecular signature to clinical significance through structured validation and collaborative integration

9.2 Summary of Contribution of Knowledge

This thesis provides several distinct contributions across clinical, translational, and infrastructural research domains:

- First nationwide assessment of *BRCA1/2* mutation prevalence and clinical impact in Irish OC, demonstrating a 20% rate of clinically actionable variants and identifying demographic and survival associations.
- Characterisation of the clinicopathologic and genetic landscape of young-onset OC in Ireland, identifying unique disease features and informing future testing strategies.
- Economic and clinical justification for mainstreaming genetic testing and improving testing pathways in the Irish healthcare setting.
- Infrastructure and Research System Contributions
- Conceptualisation, design, and launch of the CAmPaiGn pilot registry, Ireland's first integrated registry for patients with cancer-predisposing genetic alterations.
- Establishment of a scalable, GDPR-compliant data architecture linking clinical, molecular, and patient-reported outcomes, with embedded mechanisms for consent, recontact, and data sharing.
- Implementation of genuine Patient and Public Involvement (PPI) at all stages of research design, ensuring ethical integrity, governance transparency, and long-term stakeholder ownership.
- Provision of a replicable model for registry–biobank integration to support molecular validation studies nationally and across Europe.
- Development and validation of clinically relevant, pharmacokinetically informed chemotherapy- and PARPi-resistant OC models.
- Identification of key miRNAs (*miR-652-3p*, *miR-3613*, *miR-27b-3p*) and circRNAs (e.g., *hsa_circ_400223*, *hsa_circ_008026*, *hsa_circ_025636*) associated with *BRCA* status and therapeutic resistance.
- Demonstration of GO and KEGG pathway enrichment implicating chromatin organisation, intracellular structure, and protein modification as mechanisms underpinning resistance.
- Establishment of a resource for future biomarker validation through tissue microarrays and prospective biospecimen collection.

- Integration of *in silico*, *in vitro*, and clinical registry methodologies into a unified translational framework.
- Demonstration of how discovery science can directly inform clinical infrastructure and vice versa.
- Delivery of a blueprint for sustainable, inclusive, and scalable research platforms capable of supporting multicentre collaborations and national policy development in hereditary and molecular oncology.

9.3 Future Directions

Future Works: Chapter One Results

Future research should continue to build on this study by systematically adding real-world data from Irish patients with cancer-predisposing genetic alterations. While this chapter has focused on *BRCA1/2* in OC, further work should extend to other disease contexts where *BRCA* mutations are relevant, including prostate, pancreatic, and breast cancers, thereby providing a more comprehensive picture of hereditary cancer predisposition within the Irish population. In parallel, alignment with international reporting standards will be essential. Specifically, the integration of HRD scores into routine reporting would add critical information on therapeutic eligibility, particularly in the context of PARP inhibitor use. An amendment to the existing ethics approval is currently underway to address this gap.

Beyond *BRCA* and HRD, future efforts should systematically capture additional cancer-predisposing genetic alterations. Comprehensive reporting across multiple syndromes will enhance understanding of their prevalence, penetrance, and clinical outcomes. Achieving this will require more harmonised and structured data collection, as highlighted in subsequent chapters describing the development of the pilot registry at St. James's Hospital. Expansion of such initiatives, underpinned by patient and public involvement (PPI), will be critical in providing the foundation to scale towards a sustainable national registry.

There also remains significant work to be done in understanding cancer predisposition in young people. While this study has provided new insights into young-onset OC, there is a broader need to examine cancer susceptibility across adolescent and young adult (AYA) populations. Colorectal cancer has now emerged as a leading cause of cancer-related death in individuals under 40, underscoring the urgency of developing tailored prevention and early detection strategies for this group. Addressing these gaps will require integration of genomic testing, long-term follow-up, and cross-disease registries that include young patients across multiple cancer types.

Taken together, these directions represent essential next steps in strengthening Ireland's capacity to generate high-quality, population-level data on cancer-predisposing genetic alterations. Such efforts will not only inform national policy and clinical guidelines but will also position Ireland to contribute meaningfully to international collaborations in hereditary cancer research.

Future Works: Chapter Two Results

The pilot study successfully established proof of concept and feasibility for the creation of a national registry for cancer-predisposing genetic alterations. This initial phase demonstrated the potential of such a registry to collect, store, and analyse valuable data, providing a credible foundation for further development. The pilot established governance structures, data flows, recruitment processes, and a Patient and Public Involvement (PPI) programme that defined user priorities. Together, these elements constitute research infrastructure with the capacity to scale.

The next phase should move from single-site feasibility to a national framework. Multi-site participation within the HSE should be enabled using a single data dictionary, standard operating procedures, and data sharing agreements. Interoperability work should map core fields to hospital and laboratory systems so that genetics reports, screening activity, treatments, and outcomes can be ingested with minimal manual entry. Routine quality indicators should be tracked, including consent rate, baseline completeness, timeliness of entry, annual update rate, and query resolution time. Subject to approvals, linkage with national datasets, including the National Cancer Registry, should be pursued to report incident cancers, procedures, and survival. A standing PPI advisory panel with defined terms of reference should inform priorities, communications, and access policies. Regular participant updates should be delivered by email and through annual public reporting, while optional consent for recontact could support trial matching and translational sub-studies.

Moving forward, one of the primary goals is to continue to engage with the National Cancer Control Programme (NCCP) to align the registry's objectives with the strategic aims outlined in the National Cancer Strategy and the National Genomics Strategy. These documents emphasize the importance of building robust national data infrastructure to support the future of cancer care in Ireland, and the creation of a registry for cancer-predisposing genetic alterations represents a critical step in realizing this vision. Continued engagement with government and other stakeholders will be essential in securing financial support, resources, and political will to sustain and expand this initiative.

Governance will require clear accountability. One option is a dedicated not-for-profit body to steward the registry, similar to successful Irish disease-specific charities. A charity model would provide transparent financial oversight, independent boards with patient representation, an independent data access committee, and annual public reporting, while operational activity remains embedded in clinical and academic partners through formal agreements.

While the collection of demographic and clinical data forms the foundation of the registry, its long-term value lies in supporting integrated translational research. The inclusion of diagnostic and

prognostic biomarker data will be essential to enable precision oncology approaches. Over time, the dataset could support computational models capable of analysing large-scale genomic information to identify clinically actionable patterns. These in silico approaches, when combined with basic and translational research, can aid in predicting treatment response and in guiding the development of novel therapeutic strategies. Annual reporting will play a central role in disseminating findings to healthcare providers, researchers, and policymakers, informing decision-making processes related to prevention, screening, and treatment strategies for individuals with cancer-predisposing genetic alterations.

Ongoing engagement with PPI groups will remain integral to the registry's success. Regular feedback from patients, advocacy groups, and the wider public will help ensure the registry remains relevant, inclusive, and patient-centred. This dynamic approach will allow the registry to adapt to the evolving landscape of cancer genetics and healthcare needs. In parallel, collaboration with technology developers will be important to create user-friendly interfaces that prioritise accessibility, ease of use, and seamless integration with healthcare systems. Such innovations will enhance data entry, management, and analysis, thereby maximising the registry's impact for both clinical practice and research.

In conclusion, the pilot has provided proof of feasibility and established the foundations necessary for scale. The next step is to seek larger awards to support a national registry, pursued either through a charity-led structure or institutional sponsorship, aligned with national genomics policy and strategy.

Future Works: Chapter Three Results

The in vitro analyses have shown statistically significant differential expression of miRNAs such as miR_652-3p, miR_3613, miR_27b-3p, miR_205-5p, and miR_590-3p in BRCA mutant OC cell lines compared to control. Still, additional studies are required to establish their clinical relevance. While in silico analyses using large databases are useful for identifying potential prognostic biomarkers, these miRNAs must undergo rigorous validation in patient samples to ensure their reliability and applicability in clinical settings. Key steps will include analysing these miRNA signatures in larger, more diverse patient cohorts to assess their correlation with BRCA mutation status and OC.

Future mechanistic studies will need to explore the direct interactions between BRCA mutations and miRNA expression, focusing on how BRCA status might modulate the activity of specific miRNAs. This will help clarify whether these miRNAs act as downstream effectors of BRCA-driven tumour biology. Functional studies using knockout and overexpression models of BRCA-mutant and wildtype OC cells will help elucidate the role of these miRNAs in tumour growth, metastasis, and response to chemotherapy and targeted therapies. For those miRNAs that have shown statistical significance, in vivo models could be used to assess their influence within the tumour microenvironment, providing a deeper understanding of how these miRNAs may contribute to the progression of OC in the context of BRCA mutations.

Lastly, should a miRNA be validated in a large clinical sample, the development of standardized protocols for detecting these miRNAs in biofluids, such as serum or plasma, could facilitate non-invasive biomarker assays. These assays would enable more accurate identification of OC patients with specific BRCA statuses, support early diagnosis, and guide personalized treatment strategies, ultimately advancing precision oncology in OC.

Future Works: Chapter Four Results

Future studies have the potential to expand beyond established HGSOC cell lines to include patient-derived organoids and xenografts, which better capture tumour heterogeneity and stromal interactions. CRISPR-engineered isogenic models may also be useful for addressing specific mechanistic questions in BRCA-associated biology. Validation across larger and more diverse cell line panels will strengthen the reliability of circRNA findings and improve translational relevance.

Future studies could also aim to directly interrogate the role of candidate circRNAs through gain- and loss-of-function approaches. Stable overexpression can be achieved using circRNA expression vectors, which incorporate back-splice junctions to ensure circularisation and allow long-term expression in cultured cells. This would enable the creation of isogenic models where individual circRNAs can be systematically examined for their impact on proliferation, DNA repair capacity, and drug response. Conversely, silencing strategies using siRNAs or CRISPR/Cas13-based methods could complement overexpression systems to define the functional necessity of these circRNAs. Together, these approaches would provide mechanistic evidence linking circRNA dysregulation to BRCA-associated phenotypes in HGSOC.

KEGG pathway analysis identified enrichment in DNA repair, proliferation, and adhesion pathways, all directly relevant to HGSOC biology. These findings highlight areas for future functional validation. For example, homologous recombination could be examined through functional assays such as RAD51 foci formation or DNA fibre analysis, and genomic scar testing using assays such as Myriad HRD applied to cell line DNA, while proliferation can be assessed using BrdU incorporation, colony formation, and cell cycle profiling. Adhesion and migration might be explored through focal adhesion staining, transwell assay, or spheroid attachment models. Some of these approaches are introduced in the following chapter, where resistant models are developed and BrdU assays and cell counting are applied to examine proliferation.

Technical challenges were addressed in this study using rigorous detection methods, divergent primers and appropriate negative controls. Future work should now focus on expanding patient recruitment to build larger, well-powered clinical cohorts, and extending validation to additional *BRCA*-relevant tumour types such as breast, prostate, and pancreatic cancers, where PARPi are clinically actionable. Selected circRNAs identified in this study, such as circRNA_025636, should be prioritised for further exploration to clarify its potential as biomarkers of BRCA status. This could be achieved by validating their expression in larger patient cohorts and testing their functional impact through overexpression or knockdown experiments in relevant cell line models.

Future Works: Chapter Results Five

Future work should aim to expand the repertoire of resistant OC models across distinct *BRCA* backgrounds, enabling direct comparison of resistance trajectories to platinum-based chemotherapy and PARPi. To achieve this, stringent measures for contamination control, routine authentication, and careful passage management will be essential to avoid the challenges of cross-contamination and phenotypic drift encountered in this study. A broader set of validated models would provide a stronger foundation for identifying genotype-specific patterns of resistance.

Beyond model generation, mechanistic studies will be important to clarify the role of stemness markers and drug transporters in resistance. This will involve moving beyond RNA-level profiling to protein-level validation, incorporating techniques such as western blotting, ELISA, and immunofluorescence. Developing proficiency in these approaches will strengthen the capacity to interrogate molecular mechanisms directly and provide a more comprehensive picture of how resistance emerges and is sustained.

Future directions should also include the use of targeted perturbation strategies, such as siRNA knockdown or CRISPR-based editing, to determine whether pathways implicated in stemness or efflux play a causal role in the resistant phenotype. Integrating these mechanistic studies with broader omics approaches, including transcriptomic and epigenomic profiling, would generate a multidimensional understanding of resistance biology.

Ultimately, complementing in vitro experiments with more complex patient-derived models, such as xenografts or organoids, will be critical for validating findings in a clinically relevant context.

Together, these approaches would advance both technical skills and biological insight, bridging the gap between laboratory observations and translational application in OC.

Future Works: Chapter Six Results

Further validation in patient material is a priority. Banked patient derived RNA collected at defined clinical time points (i.e. baseline, on-treatment, and at progression etc), can provide an opportunity to test whether the leading candidates (for example, circRNA 85803 and circRNA 3258) track treatment response and predict PFS and OS. In parallel, RT qPCR or ddPCR assays require validation for use in circRNA studies and optimisation for FFPE tissue and plasma to assess feasibility as both tissue and liquid-biopsy biomarkers.

Mechanistic studies are also needed to define function. Confirmation of circularity and stability (RNase R and transcription-block assays), loss- and gain-of-function experiments in parental and resistant models, and rescue studies should determine effects on drug sensitivity, proliferation, and cell-cycle control. Subcellular localisation studies using fractionation or RNA FISH, and RNA–protein interaction assays could confirm the signals from the computational analysis (i.e. The GO analysis). Replication in additional HGSOC lines, patient-derived organoids, and in vivo models will clarify context dependence and BRCA/HRD specificity. Prospective correlative studies embedded within chemotherapy and PARPi trials may help to determine whether circRNA panels add value beyond established clinico-genomic predictors for treatment selection or early progression monitoring.

9.4 Overall Conclusion

This thesis has explored the genomic, molecular, and infrastructural dimensions of hereditary and treatment-resistant ovarian cancer within the Irish context. The work combined epidemiological, translational and laboratory approaches to address a fundamental gap in national understanding of genetic cancer predisposition and its clinical impact. Across the programme of work, three major contributions were achieved:

1. a comprehensive characterisation of *BRCA1/2* prevalence and clinicopathological correlates in Irish OC;
2. the design and implementation of the CAmPaiGn pilot registry—the first structured effort to establish a national framework for the systematic collection of data on cancer-predisposing genetic alterations; and
3. novel molecular insights into the role of miRNA and circRNA networks in *BRCA*-associated and treatment-resistant OC

Together, these components provide an integrated foundation for precision oncology in Ireland. The thesis demonstrates that translational discovery cannot advance without parallel investment in digital and data infrastructures capable of supporting longitudinal, population-level genomic research. The CAmPaiGn pilot registry exemplifies a feasible, ethically robust, and publicly supported model through which clinical, molecular, and patient-reported data can be linked to improve cancer prevention and care.

From a scientific perspective, this work identified and validated several miRNA and circRNA candidates associated with *BRCA*-linked and treatment-resistant ovarian cancer models. Specifically, miR-652-3p, miR-27b-3p, and miR-3613 were confirmed as differentially expressed across *BRCA1*, *BRCA2*, and wildtype cell lines, supporting their potential role in homologous recombination and DNA repair regulation. Parallel circRNA profiling revealed distinct signatures in *BRCA*-mutant and resistant models, with circ_025636 emerging as a candidate biomarker of therapeutic response. Together, these findings provide a molecular rationale for further biomarker development and functional validation in patient cohorts. They also illustrate the translational value of integrating in silico prediction with in vitro and clinical model systems to define molecular mechanisms of resistance and guide precision treatment strategies in ovarian cancer.

Overall, this body of work contributes new empirical data, infrastructure, and frameworks necessary to advance genomic medicine in Ireland. It establishes the groundwork for future national registry expansion, international collaboration, and validation of molecular biomarkers in patient cohorts. The collective impact of this research lies not only in its scientific outputs but also in its

demonstration that coordinated, patient-centred, and data-driven approaches can bridge the gap between discovery and implementation in hereditary and treatment-resistant OC.

10 References

1. Alexandrova E, Pecoraro G, Sellitto A, Melone V, Ferravante C, Rocco T, et al. An Overview of Candidate Therapeutic Target Genes in Ovarian Cancer. *Cancers (Basel)*. 2020;12(6).
2. Akter S, Rahman MA, Hasan MN, Akhter H, Noor P, Islam R, et al. Recent Advances in Ovarian Cancer: Therapeutic Strategies, Potential Biomarkers, and Technological Improvements. *Cells*. 2022;11(4).
3. Konstantinopoulos PA, Lheureux S, Moore KN. PARP Inhibitors for Ovarian Cancer: Current Indications, Future Combinations, and Novel Assets in Development to Target DNA Damage Repair. *American Society of Clinical Oncology Educational Book*. 2020(40):e116-e31.
4. Integrated genomic analyses of ovarian carcinoma. *Nature*. 2011;474(7353):609-15.
5. Nesic K, Wakefield M, Kondrashova O, Scott CL, McNeish IA. Targeting DNA repair: the genome as a potential biomarker. *J Pathol*. 2018;244(5):586-97.
6. Konstantinopoulos PA, Matulonis UA. Targeting DNA Damage Response and Repair as a Therapeutic Strategy for Ovarian Cancer. *Hematol Oncol Clin North Am*. 2018;32(6):997-1010.
7. Glajzer J, Castillo-Tong DC, Richter R, Vergote I, Kulbe H, Vanderstichele A, et al. Impact of BRCA Mutation Status on Tumor Dissemination Pattern, Surgical Outcome and Patient Survival in Primary and Recurrent High-Grade Serous Ovarian Cancer: A Multicenter Retrospective Study by the Ovarian Cancer Therapy-Innovative Models Prolong Survival (OCTIPS) Consortium. *Annals of Surgical Oncology*. 2023;30(1):35-45.
8. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib Maintenance Therapy in Platinum-Sensitive Relapsed Ovarian Cancer. *New England Journal of Medicine*. 2012;366(15):1382-92.
9. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N Engl J Med*. 2018;379(26):2495-505.
10. Ray-Coquard I, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, et al. Olaparib plus Bevacizumab as First-Line Maintenance in Ovarian Cancer. *N Engl J Med*. 2019;381(25):2416-28.
11. Monk BJ, Parkinson C, Lim MC, O'Malley DM, Oaknin A, Wilson MK, et al. A Randomized, Phase III Trial to Evaluate Rucaparib Monotherapy as Maintenance Treatment in Patients With Newly Diagnosed Ovarian Cancer (ATHENA-MONO/GOG-3020/ENGOT-ov45). *J Clin Oncol*. 2022;40(34):3952-64.
12. Kristeleit R, Lisyanskaya A, Fedenko A, Dvorkin M, de Melo AC, Shparyk Y, et al. Rucaparib versus standard-of-care chemotherapy in patients with relapsed ovarian cancer and a deleterious BRCA1 or BRCA2 mutation (ARIEL4): an international, open-label, randomised, phase 3 trial. *Lancet Oncol*. 2022;23(4):465-78.
13. Mirza MR, Coleman RL, González-Martín A, Moore KN, Colombo N, Ray-Coquard I, Pignata S. The forefront of ovarian cancer therapy: update on PARP inhibitors. *Ann Oncol*. 2020;31(9):1148-59.
14. Gadducci A, Guarneri V, Peccatori FA, Ronzino G, Scandurra G, Zamagni C, et al. Current strategies for the targeted treatment of high-grade serous epithelial ovarian cancer and relevance of BRCA mutational status. *J Ovarian Res*. 2019;12(1):9.
15. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018. *CA Cancer J Clin*. 2018;68(4):284-96.
16. Registry NC. Cancer Factsheet Ovary 2018 [Available from: <https://www.ncri.ie/sites/ncri/files/factsheets/Factsheet%20Ovary.pdf>].
17. Mangili G, Sigismondi C, Gadducci A, Cormio G, Scillo P, Tateo S, et al. Outcome and risk factors for recurrence in malignant ovarian germ cell tumors: a MITO-9 retrospective study. *Int J Gynecol Cancer*. 2011;21(8):1414-21.
18. Gershenson DM. Management of ovarian germ cell tumors. *J Clin Oncol*. 2007;25(20):2938-43.
19. Berek JS, Renz M, Kehoe S, Kumar L, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum: 2021 update. *Int J Gynaecol Obstet*. 2021;155 Suppl 1(Suppl 1):61-85.

20. Webb PM, Jordan SJ. Epidemiology of epithelial ovarian cancer. *Best Pract Res Clin Obstet Gynaecol.* 2017;41:3-14.
21. Institute NC. Cancer stat facts: ovarian cancer. . [internet publication]. 2022.
22. Cancer IAFRo. Ovary Globocan 2020 2023 [Available from: <https://gco.iarc.fr/today/data/factsheets/cancers/25-Ovary-fact-sheet.pdf>.
23. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.* 2021;71(3):209-49.
24. Coalition WOC. Ovarian Cancer Key Stats* 2022 [9/20/2024]. Available from: <https://worldovariancancercoalition.org/about-ovarian-cancer/key-stats/>.
25. Stratton JF, Pharoah P, Smith SK, Easton D, Ponder BA. A systematic review and meta-analysis of family history and risk of ovarian cancer. *Br J Obstet Gynaecol.* 1998;105(5):493-9.
26. Kazerouni N, Greene MH, Lacey JV, Jr., Mink PJ, Schairer C. Family history of breast cancer as a risk factor for ovarian cancer in a prospective study. *Cancer.* 2006;107(5):1075-83.
27. Lisio MA, Fu L, Goyeneche A, Gao ZH, Telleria C. High-Grade Serous Ovarian Cancer: Basic Sciences, Clinical and Therapeutic Standpoints. *Int J Mol Sci.* 2019;20(4).
28. Boyd J. Specific keynote: hereditary ovarian cancer: what we know. *Gynecol Oncol.* 2003;88(1 Pt 2):S8-10; discussion S1-3.
29. Kauff ND, Satagopan JM, Robson ME, Scheuer L, Hensley M, Hudis CA, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. *N Engl J Med.* 2002;346(21):1609-15.
30. Lu KH, Daniels M. Endometrial and ovarian cancer in women with Lynch syndrome: update in screening and prevention. *Familial Cancer.* 2013;12(2):273-7.
31. Pearce CL, Templeman C, Rossing MA, Lee A, Near AM, Webb PM, et al. Association between endometriosis and risk of histological subtypes of ovarian cancer: a pooled analysis of case-control studies. *Lancet Oncol.* 2012;13(4):385-94.
32. Oncology NCPGi. Ovarian Cancer Including Fallopian Tube Cancer and Primary Peritoneal Cancer. 2023 (Version 1.2023).
33. Zhou Z, Zeng F, Yuan J, Tang J, Colditz GA, Tworoger SS, et al. Pelvic inflammatory disease and the risk of ovarian cancer: a meta-analysis. *Cancer Causes Control.* 2017;28(5):415-28.
34. Rasmussen CB, Kjaer SK, Albieri V, Bandera EV, Doherty JA, Høgdall E, et al. Pelvic Inflammatory Disease and the Risk of Ovarian Cancer and Borderline Ovarian Tumors: A Pooled Analysis of 13 Case-Control Studies. *Am J Epidemiol.* 2017;185(1):8-20.
35. Fund WCR. Ovarian Cancer Statistics [Internet Page]. 2022.
36. Grossman DC, Curry SJ, Owens DK, Barry MJ, Davidson KW, Doubeni CA, et al. Screening for Ovarian Cancer: US Preventive Services Task Force Recommendation Statement. *Jama.* 2018;319(6):588-94.
37. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin.* 2017;67(2):93-9.
38. Matz M, Coleman MP, Sant M, Chirlaque MD, Visser O, Gore M, Allemani C. The histology of ovarian cancer: worldwide distribution and implications for international survival comparisons (CONCORD-2). *Gynecol Oncol.* 2017;144(2):405-13.
39. Kurman RJ, Shih Ie M. The Dualistic Model of Ovarian Carcinogenesis: Revisited, Revised, and Expanded. *Am J Pathol.* 2016;186(4):733-47.
40. Lu Z, Chen J. [Introduction of WHO classification of tumours of female reproductive organs, fourth edition]. *Zhonghua Bing Li Xue Za Zhi.* 2014;43(10):649-50.
41. Mayr D, Schmoeckel E, Höhn AK, Hiller GGR, Horn LC. [Current WHO classification of the female genitals : Many new things, but also some old]. *Pathologe.* 2021;42(3):259-69.
42. Prat J, D'Angelo E, Espinosa I. Ovarian carcinomas: at least five different diseases with distinct histological features and molecular genetics. *Hum Pathol.* 2018;80:11-27.

43. Prat J. New insights into ovarian cancer pathology. *Ann Oncol.* 2012;23 Suppl 10:x111-7.
44. Ha HI, Kim JH, Lim J, Song YJ, Won YJ, Lim MC. Incidence and treatment outcomes of ovarian carcinosarcoma from the national cancer registry of Korea. *J Gynecol Oncol.* 2024;35(1):e31.
45. Chen Q, Wang S, Lang J-H. Clinical characteristics and prognostic factors of stage IC ovarian clear cell carcinoma: a Surveillance, Epidemiology, and End Results (SEER) analysis. *Archives of Gynecology and Obstetrics.* 2021;304(2):521-9.
46. McCabe A, Zaheed O, McDade SS, Dean K. Investigating the suitability of in vitro cell lines as models for the major subtypes of epithelial ovarian cancer. *Frontiers in Cell and Developmental Biology.* 2023;11.
47. Hollis RL, Croy I, Churchman M, Bartos C, Rye T, Gourley C, Herrington CS. Ovarian carcinosarcoma is a distinct form of ovarian cancer with poorer survival compared to tubo-ovarian high-grade serous carcinoma. *Br J Cancer.* 2022;127(6):1034-42.
48. Jones S, Wang TL, Shih Ie M, Mao TL, Nakayama K, Roden R, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. *Science.* 2010;330(6001):228-31.
49. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD. Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer. *Cancer Discov.* 2015;5(11):1137-54.
50. Lockley M, Stoneham SJ, Olson TA. Ovarian cancer in adolescents and young adults. *Pediatr Blood Cancer.* 2019;66(3):e27512.
51. Irshaid L, Costigan DC, Dong F, Matulonis UA, Nucci MR, Kolin DL. Molecular Landscape of Mullerian Clear Cell Carcinomas Identifies The Cancer Genome Atlas-like Prognostic Subgroups. *Mod Pathol.* 2023;36(5):100123.
52. González-Martín A, Harter P, Leary A, Lorusso D, Miller RE, Pothuri B, et al. Newly diagnosed and relapsed epithelial ovarian cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol.* 2023;34(10):833-48.
53. Ledermann JA, Matias-Guiu X, Amant F, Concin N, Davidson B, Fotopoulou C, et al. ESGO-ESMO-ESP consensus conference recommendations on ovarian cancer: pathology and molecular biology and early, advanced and recurrent disease. *Ann Oncol.* 2024;35(3):248-66.
54. Bouchard-Fortier G, Panzarella T, Rosen B, Chapman W, Gien LT. Endometrioid Carcinoma of the Ovary: Outcomes Compared to Serous Carcinoma After 10 Years of Follow-Up. *J Obstet Gynaecol Can.* 2017;39(1):34-41.
55. Vang R, Shih Ie M, Kurman RJ. Ovarian low-grade and high-grade serous carcinoma: pathogenesis, clinicopathologic and molecular biologic features, and diagnostic problems. *Adv Anat Pathol.* 2009;16(5):267-82.
56. La Vecchia C. Ovarian cancer: epidemiology and risk factors. *Eur J Cancer Prev.* 2017;26(1):55-62.
57. De Nonneville A, Zemmour C, Frank S, Joly F, Ray-Coquard I, Costaz H, et al. Clinicopathological characterization of a real-world multicenter cohort of endometrioid ovarian carcinoma: Analysis of the French national ESME-Unicancer database. *Gynecol Oncol.* 2021;163(1):64-71.
58. Qiu Y, Chen Y, Agbede O, Eshaghi E, Peng C. Circular RNAs in Epithelial Ovarian Cancer: From Biomarkers to Therapeutic Targets. *Cancers (Basel).* 2022;14(22).
59. DiSilvestro P, Banerjee S, Colombo N, Scambia G, Kim BG, Oaknin A, et al. Overall Survival With Maintenance Olaparib at a 7-Year Follow-Up in Patients With Newly Diagnosed Advanced Ovarian Cancer and a BRCA Mutation: The SOLO1/GOG 3004 Trial. *J Clin Oncol.* 2023;41(3):609-17.
60. Ledermann JA, Drew Y, Kristeleit RS. Homologous recombination deficiency and ovarian cancer. *European Journal of Cancer.* 2016;60:49-58.
61. Choi HJ, Armaiz Pena GN, Pradeep S, Cho MS, Coleman RL, Sood AK. Anti-vascular therapies in ovarian cancer: moving beyond anti-VEGF approaches. *Cancer Metastasis Rev.* 2015;34(1):19-40.
62. Network NCC. NCCN Guidelines Version 1.2023 Ovarian Cancer. Online 2023.

63. Elattar A, Bryant A, Winter-Roach BA, Hatem M, Naik R. Optimal primary surgical treatment for advanced epithelial ovarian cancer. *Cochrane Database Syst Rev*. 2011;2011(8):Cd007565.
64. Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. *J Clin Oncol*. 2002;20(5):1248-59.
65. von Georgi R, Franke FE, Münstedt K. Influence of surgery and postoperative therapy and tumor characteristics on patient prognosis in advanced ovarian carcinomas. *Eur J Obstet Gynecol Reprod Biol*. 2003;111(2):189-96.
66. Alberts DS, Green S, Hannigan EV, O'Toole R, Stock-Novack D, Anderson P, et al. Improved therapeutic index of carboplatin plus cyclophosphamide versus cisplatin plus cyclophosphamide: final report by the Southwest Oncology Group of a phase III randomized trial in stages III and IV ovarian cancer. *J Clin Oncol*. 1992;10(5):706-17.
67. Rowinsky EK, Onetto N, Canetta RM, Arbuck SG. Taxol: the first of the taxanes, an important new class of antitumor agents. *Semin Oncol*. 1992;19(6):646-62.
68. McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. *N Engl J Med*. 1996;334(1):1-6.
69. Bookman MA, Greer BE, Ozols RF. Optimal therapy of advanced ovarian cancer: carboplatin and paclitaxel versus cisplatin and paclitaxel (GOG158) and an update on GOG0182-ICON5. *Int J Gynecol Cancer*. 2003;13 Suppl 2:149-55.
70. Clamp AR, James EC, McNeish IA, Dean A, Kim J-W, O'Donnell DM, et al. Weekly dose-dense chemotherapy in first-line epithelial ovarian, fallopian tube, or primary peritoneal carcinoma treatment (ICON8): primary progression free survival analysis results from a GCIg phase 3 randomised controlled trial. *The Lancet*. 2019;394(10214):2084-95.
71. Kaufman B, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, Balmaña J, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. *J Clin Oncol*. 2015;33(3):244-50.
72. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol*. 2014;15(8):852-61.
73. Caruso G, Tomao F, Parma G, Lapresa M, Multinu F, Palaia I, et al. Poly (ADP-ribose) polymerase inhibitors (PARPi) in ovarian cancer: lessons learned and future directions. *Int J Gynecol Cancer*. 2023.
74. FDA. FDA approved olaparib (LYNPARZA, AstraZeneca Pharmaceuticals LP) for the maintenance treatment of adult patients with deleterious or suspected deleterious germline or somatic BRCA-mutated (gBRCAm or sBRCAm) advanced epithelial ovarian, fallopian tube or primary peritoneal cancer who are in complete or partial response to first-line platinum-based. 2018.
75. González-Martín A, Pothuri B, Vergote I, DePont Christensen R, Graybill W, Mirza MR, et al. Niraparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N Engl J Med*. 2019;381(25):2391-402.
76. FDA. FDA approves niraparib for first-line maintenance of advanced ovarian cancer. 2020.
77. SUMMARY: REVISIONS TO FDA APPROVALS FOR PARP INHIBITORS IN THE MANAGEMENT OF OVARIAN CANCER, (Dec 9, 2022).
78. Sánchez-Lorenzo L, Salas-Benito D, Villamayor J, Patiño-García A, González-Martín A. The BRCA Gene in Epithelial Ovarian Cancer. *Cancers (Basel)*. 2022;14(5).
79. Jenner ZB, Sood AK, Coleman RL. Evaluation of rucaparib and companion diagnostics in the PARP inhibitor landscape for recurrent ovarian cancer therapy. *Future Oncol*. 2016;12(12):1439-56.
80. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med*. 2012;366(15):1382-92.

81. Poveda A, Floquet A, Ledermann JA, Asher R, Penson RT, Oza AM, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a final analysis of a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol.* 2021;22(5):620-31.
82. Ray-Coquard I, Leary A, Pignata S, Cropet C, González-Martín A, Marth C, et al. Olaparib plus bevacizumab first-line maintenance in ovarian cancer: final overall survival results from the PAOLA-1/ENGOT-ov25 trial. *Annals of Oncology.* 2023;34(8):681-92.
83. Registry NC. Cancer in Ireland 1994-2021: Annual Statistical report of the National Cancer Registry. Building 6800

Cork Airport Business Park

Kinsale Road, Cork T12 CDF7: National Cancer Registry; 2023.

84. (NCRI) NCRI. Cancer Factsheet Ovary (ICD-10 C56). 2020.
85. Association EFoPI. OVARIAN CANCER 2024 [Available from: <https://www.efpia.eu/publications/cancer-comparator-report/cancer-types/ovarian-cancer/>].
86. Ireland NCR. CANCER IN IRELAND 1994-2018 WITH ESTIMATES FOR 2018-2020: Annual Report. 2020.
87. O'Reilly DE, Dooley L, Watson GA, Clarke R, Nolan A, Nolan C, et al. Uptake of BRCA1/BRCA2 predictive genetic testing in an Irish population is low: a missed opportunity. *Ir J Med Sci.* 2023;192(4):1607-11.
88. Kalachand R. Cancer Trials Ireland opens study to enable upfront and streamlined germline and somatic BRCA1/2 mutation testing in women with ovarian cancer 2019.
89. McVeigh TP, Sweeney KJ, Brennan DJ, McVeigh UM, Ward S, Strydom A, et al. A pilot study investigating feasibility of mainstreaming germline BRCA1 and BRCA2 testing in high-risk patients with breast and/or ovarian cancer in three tertiary Cancer Centres in Ireland. *Fam Cancer.* 2023;22(2):135-49.
90. HSE. National Strategy for Accelerating Genetic and Genomic Medicine in Ireland. In: Health Do, editor. 2022.
91. Authority HlaQ. National Renal Transplant Registry 2022 [Available from: <https://www.hiqa.ie/areas-we-work/health-information/data-collections/national-renal-transplant-registry#:~:text=This%20database%20is%20a%20renal,kidney%20disease%2C%20length%20of%20stay>].
92. HSE. National Framework for the Integrated Prevention and Management of Chronic Disease in Ireland 2020 – 2025 2020 [Available from: <https://www.hse.ie/eng/about/who/cspd/icp/chronic-disease/documents/the-chronic-disease-integrated-care-programme-10-step-guide.pdf>].
93. HSE. European Reference Networks (ERNs) for Rare Diseases 2024 [20/09/2024].
94. Health Do. National Strategy for Accelerating Genetic and Genomic Medicine in Ireland. In: H.S.E., editor. 2022.
95. Seoighe C, Bracken AP, Buckley P, Doran P, Green R, Healy S, et al. The future of genomics in Ireland - focus on genomics for health. *HRB Open Res.* 2020;3:89.
96. HSE. REVIEW OF THE CLINICAL GENETICS MEDICAL WORKFORCE IN IRELAND. In: Planning NDT, editor. 2019.
97. King MC. "The race" to clone BRCA1. *Science.* 2014;343(6178):1462-5.
98. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips K-A, Mooij TM, Roos-Blom M-J, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *JAMA.* 2017;317(23):2402-16.
99. McNevin CS, Cadoo K, Baird AM, Murchan P, Sheils O, McDermott R, Finn S. Pathogenic BRCA Variants as Biomarkers for Risk in Prostate Cancer. *Cancers (Basel).* 2021;13(22).

100. McNevin CS, Cadoo K, Baird AM, Finn SP, McDermott R. PARP Inhibitors in Advanced Prostate Cancer in Tumors with DNA Damage Signatures. *Cancers (Basel)*. 2022;14(19).
101. Finn K, Lowndes NF, Grenon M. Eukaryotic DNA damage checkpoint activation in response to double-strand breaks. *Cell Mol Life Sci*. 2012;69(9):1447-73.
102. Oh JM, Myung K. Crosstalk between different DNA repair pathways for DNA double strand break repairs. *Mutat Res Genet Toxicol Environ Mutagen*. 2022;873:503438.
103. Stok C, Kok YP, van den Tempel N, van Vugt M. Shaping the BRCAness mutational landscape by alternative double-strand break repair, replication stress and mitotic aberrancies. *Nucleic Acids Res*. 2021;49(8):4239-57.
104. Neiger HE, Siegler EL, Shi Y. Breast Cancer Predisposition Genes and Synthetic Lethality. *Int J Mol Sci*. 2021;22(11).
105. Lundin C, Erixon K, Arnaudeau C, Schultz N, Jenssen D, Meuth M, Helleday T. Different Roles for Nonhomologous End Joining and Homologous Recombination following Replication Arrest in Mammalian Cells. *Molecular and Cellular Biology*. 2002;22(16):5869-78.
106. Schlacher K, Wu H, Jasin M. A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. *Cancer Cell*. 2012;22(1):106-16.
107. Prakash R, Zhang Y, Feng W, Jasin M. Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. *Cold Spring Harb Perspect Biol*. 2015;7(4):a016600.
108. Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, et al. Genomic correlates of clinical outcome in advanced prostate cancer. *Proceedings of the National Academy of Sciences*. 2019;116(23):11428-36.
109. Pearce CL, Stram DO, Ness RB, Stram DA, Roman LD, Templeman C, et al. Population distribution of lifetime risk of ovarian cancer in the United States. *Cancer Epidemiol Biomarkers Prev*. 2015;24(4):671-6.
110. Nesic K, Wakefield M, Kondrashova O, Scott CL, McNeish IA. Targeting DNA repair: the genome as a potential biomarker. *The Journal of Pathology*. 2018;244(5):586-97.
111. Norquist BM, Harrell MI, Brady MF, Walsh T, Lee MK, Gulsuner S, et al. Inherited Mutations in Women With Ovarian Carcinoma. *JAMA Oncol*. 2016;2(4):482-90.
112. Mota A, S SO, Moreno-Bueno G. Insight updating of the molecular hallmarks in ovarian carcinoma. *EJC Suppl*. 2020;15:16-26.
113. MacDonagh L, Gallagher MF, Ffrench B, Gasch C, Gray SG, Reidy M, et al. MicroRNA expression profiling and biomarker validation in treatment-naïve and drug resistant non-small cell lung cancer. *Transl Lung Cancer Res*. 2021;10(4):1773-91.
114. Ferneza S, Fetsych M, Shuliak R, Makukh H, Volodko N, Yarema R, Fetsych T. Clinical significance of microRNA-200 and let-7 families expression assessment in patients with ovarian cancer. *Ecancermedicalscience*. 2021;15:1249.
115. Oliveto S, Mancino M, Manfrini N, Biffo S. Role of microRNAs in translation regulation and cancer. *World J Biol Chem*. 2017;8(1):45-56.
116. Si W, Shen J, Zheng H, Fan W. The role and mechanisms of action of microRNAs in cancer drug resistance. *Clinical Epigenetics*. 2019;11(1):25.
117. Alizamir A, Amini MA, Karbasi A, Beyrami M. MiR-4492, a New Potential MicroRNA for Cancer Diagnosis and Treatment: A Mini Review. *Chonnam Med J*. 2024;60(1):21-6.
118. Otmani K, Lewalle P. Tumor Suppressor miRNA in Cancer Cells and the Tumor Microenvironment: Mechanism of Deregulation and Clinical Implications. *Front Oncol*. 2021;11:708765.
119. Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-Mediated Gene Regulation from Common Downregulation to mRNA-Specific Upregulation. *Int J Genomics*. 2014;2014:970607.
120. Lopacinska-Jørgensen J, Oliveira D, Wayne Novotny G, Høgdall CK, Høgdall EV. Integrated microRNA and mRNA signatures associated with overall survival in epithelial ovarian cancer. *PLoS One*. 2021;16(7):e0255142.

121. Bagheri R, Ghorbian M, Ghorbian S. Tumor circulating biomarkers in colorectal cancer. *Cancer Treatment and Research Communications*. 2024;38:100787.
122. Nguyen VHL, Yue C, Du KY, Salem M, O'Brien J, Peng C. The Role of microRNAs in Epithelial Ovarian Cancer Metastasis. *Int J Mol Sci*. 2020;21(19).
123. Márton É, Lukács J, Penyige A, Janka E, Hegedüs L, Soltész B, et al. Circulating epithelial-mesenchymal transition-associated miRNAs are promising biomarkers in ovarian cancer. *J Biotechnol*. 2019;297:58-65.
124. Meng X, Joosse SA, Müller V, Trillsch F, Milde-Langosch K, Mahner S, et al. Diagnostic and prognostic potential of serum miR-7, miR-16, miR-25, miR-93, miR-182, miR-376a and miR-429 in ovarian cancer patients. *Br J Cancer*. 2015;113(9):1358-66.
125. Cui Y, Hong S, Zhu X. The Accuracy of Single MicroRNAs in Peripheral Blood to Diagnose Ovarian Cancer: An Updated Meta-Analysis. *Dis Markers*. 2020;2020:1075942.
126. Krek A, Grün D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, et al. Combinatorial microRNA target predictions. *Nat Genet*. 2005;37(5):495-500.
127. Tiberio P, Callari M, Angeloni V, Daidone MG, Appierto V. Challenges in using circulating miRNAs as cancer biomarkers. *Biomed Res Int*. 2015;2015:731479.
128. Wahab NA, Othman Z, Nasri NWM, Mokhtar MH, Ibrahim SF, Hamid AA, et al. Inhibition of miR-141 and miR-200a Increase DLC-1 and ZEB2 Expression, Enhance Migration and Invasion in Metastatic Serous Ovarian Cancer. *Int J Environ Res Public Health*. 2020;17(8).
129. Parikh A, Lee C, Joseph P, Marchini S, Baccarini A, Kolev V, et al. microRNA-181a has a critical role in ovarian cancer progression through the regulation of the epithelial-mesenchymal transition. *Nat Commun*. 2014;5:2977.
130. Visone R, Croce CM. MiRNAs and cancer. *Am J Pathol*. 2009;174(4):1131-8.
131. Kumar V, Pandey A, Arora A, Gautam P, Bisht D, Gupta S, et al. Diagnostics and Therapeutic Potential of miR-205 and miR-34a in Ovarian Cancer Management: A miRNA-Target-Based Analysis. *DNA Cell Biol*. 2023;42(3):151-62.
132. Welpöner H, Tsubulak I, Wieser V, Degasper C, Shivalingaiah G, Wenzel S, et al. The miR-34 family and its clinical significance in ovarian cancer. *J Cancer*. 2020;11(6):1446-56.
133. Hermeking H. The miR-34 family in cancer and apoptosis. *Cell Death & Differentiation*. 2010;17(2):193-9.
134. Ahmed AA, Etemadmoghadam D, Temple J, Lynch AG, Riad M, Sharma R, et al. Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary. *J Pathol*. 2010;221(1):49-56.
135. Wong MY, Yu Y, Walsh WR, Yang JL. microRNA-34 family and treatment of cancers with mutant or wild-type p53 (Review). *Int J Oncol*. 2011;38(5):1189-95.
136. De Santis C, Götte M. The Role of microRNA Let-7d in Female Malignancies and Diseases of the Female Reproductive Tract. *Int J Mol Sci*. 2021;22(14).
137. García-Vázquez R, Gallardo Rincón D, Ruiz-García E, Meneses García A, Hernández De La Cruz ON, Astudillo-De La Vega H, et al. let-7d-3p is associated with apoptosis and response to neoadjuvant chemotherapy in ovarian cancer. *Oncol Rep*. 2018;39(6):3086-94.
138. Kuang Y, Xu H, Lu F, Meng J, Yi Y, Yang H, et al. Inhibition of microRNA let-7b expression by KDM2B promotes cancer progression by targeting EZH2 in ovarian cancer. *Cancer Science*. 2021;112(1):231-42.
139. Xiao M, Cai J, Cai L, Jia J, Xie L, Zhu Y, et al. Let-7e sensitizes epithelial ovarian cancer to cisplatin through repressing DNA double strand break repair. *J Ovarian Res*. 2017;10(1):24.
140. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, et al. RAS Is Regulated by the let-7 MicroRNA Family. *Cell*. 2005;120(5):635-47.
141. Lou Y, Yang X, Wang F, Cui Z, Huang Y. MicroRNA-21 promotes the cell proliferation, invasion and migration abilities in ovarian epithelial carcinomas through inhibiting the expression of PTEN protein. *Int J Mol Med*. 2010;26(6):819-27.

142. McMillen BD, Aponte MM, Liu Z, Helenowski IB, Scholtens DM, Buttin BM, Wei JJ. Expression analysis of MIR182 and its associated target genes in advanced ovarian carcinoma. *Mod Pathol*. 2012;25(12):1644-53.
143. Li X, Dai A, Tran R, Wang J. Identifying miRNA biomarkers for breast cancer and ovarian cancer: a text mining perspective. *Breast Cancer Res Treat*. 2023;201(1):5-14.
144. Zhuang RJ, Bai XX, Liu W. MicroRNA-23a depletion promotes apoptosis of ovarian cancer stem cell and inhibits cell migration by targeting DLG2. *Cancer Biol Ther*. 2019;20(6):897-911.
145. Liu F, Zhang G, Lv S, Wen X, Liu P. miRNA-301b-3p accelerates migration and invasion of high-grade ovarian serous tumor via targeting CPEB3/EGFR axis. *J Cell Biochem*. 2019;120(8):12618-27.
146. Wang Y, Zhang X, Tang W, Lin Z, Xu L, Dong R, et al. miR-130a upregulates mTOR pathway by targeting TSC1 and is transactivated by NF- κ B in high-grade serous ovarian carcinoma. *Cell Death Differ*. 2017;24(12):2089-100.
147. Braicu OL, Budisan L, Buiga R, Jurj A, Achimas-Cadariu P, Pop LA, et al. miRNA expression profiling in formalin-fixed paraffin-embedded endometriosis and ovarian cancer samples. *Onco Targets Ther*. 2017;10:4225-38.
148. Cavallari I, Ciccarese F, Sharova E, Urso L, Raimondi V, Silic-Benussi M, et al. The miR-200 Family of microRNAs: Fine Tuners of Epithelial-Mesenchymal Transition and Circulating Cancer Biomarkers. *Cancers (Basel)*. 2021;13(23).
149. Miśkiewicz J, Mielczarek-Palacz A, Gola JM. MicroRNAs as Potential Biomarkers in Gynecological Cancers. *Biomedicines*. 2023;11(6).
150. Vilming Elgaaen B, Olstad OK, Haug KB, Brusletto B, Sandvik L, Staff AC, et al. Global miRNA expression analysis of serous and clear cell ovarian carcinomas identifies differentially expressed miRNAs including miR-200c-3p as a prognostic marker. *BMC Cancer*. 2014;14:80.
151. Zhang M, Jabbari N, Satpathy M, Matyunina LV, Wang Y, McDonald LD, McDonald JF. Sequence diverse miRNAs converge to induce mesenchymal-to-epithelial transition in ovarian cancer cells through direct and indirect regulatory controls. *Cancer Lett*. 2019;459:168-75.
152. Chen J, Wang L, Matyunina LV, Hill CG, McDonald JF. Overexpression of miR-429 induces mesenchymal-to-epithelial transition (MET) in metastatic ovarian cancer cells. *Gynecol Oncol*. 2011;121(1):200-5.
153. Mhatre A, Koroth J, Manjunath M, Kumar SS, Gawari R, Choudhary B. Multi-omics analysis of the Indian ovarian cancer cohort revealed histotype-specific mutation and gene expression patterns. *Front Genet*. 2023;14:1102114.
154. Zuberi M, Mir R, Das J, Ahmad I, Javid J, Yadav P, et al. Expression of serum miR-200a, miR-200b, and miR-200c as candidate biomarkers in epithelial ovarian cancer and their association with clinicopathological features. *Clin Transl Oncol*. 2015;17(10):779-87.
155. Mateescu B, Batista L, Cardon M, Gruosso T, de Feraudy Y, Mariani O, et al. miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response. *Nat Med*. 2011;17(12):1627-35.
156. Suo HB, Zhang KC, Zhao J. MiR-200a promotes cell invasion and migration of ovarian carcinoma by targeting PTEN. *Eur Rev Med Pharmacol Sci*. 2018;22(13):4080-9.
157. Yang D, Sun Y, Hu L, Zheng H, Ji P, Pecot CV, et al. Integrated analyses identify a master microRNA regulatory network for the mesenchymal subtype in serous ovarian cancer. *Cancer Cell*. 2013;23(2):186-99.
158. Pecot CV, Rupaimoole R, Yang D, Akbani R, Ivan C, Lu C, et al. Tumour angiogenesis regulation by the miR-200 family. *Nat Commun*. 2013;4:2427.
159. Sun Q, Zou X, Zhang T, Shen J, Yin Y, Xiang J. The role of miR-200a in vasculogenic mimicry and its clinical significance in ovarian cancer. *Gynecol Oncol*. 2014;132(3):730-8.
160. Ribatti D, Tamma R, Annese T. Epithelial-Mesenchymal Transition in Cancer: A Historical Overview. *Transl Oncol*. 2020;13(6):100773.

161. Loh CY, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, et al. The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges. *Cells*. 2019;8(10).
162. Korpál M, Lee ES, Hu G, Kang Y. The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *J Biol Chem*. 2008;283(22):14910-4.
163. Hurteau GJ, Carlson JA, Spivack SD, Brock GJ. Overexpression of the microRNA hsa-miR-200c leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. *Cancer Res*. 2007;67(17):7972-6.
164. Koutsaki M, Libra M, Spandidos DA, Zaravinos A. The miR-200 family in ovarian cancer. *Oncotarget*. 2017;8(39):66629-40.
165. Lu MH, Huang CC, Pan MR, Chen HH, Hung WC. Prospero homeobox 1 promotes epithelial-mesenchymal transition in colon cancer cells by inhibiting E-cadherin via miR-9. *Clin Cancer Res*. 2012;18(23):6416-25.
166. Zhou B, Xu H, Xia M, Sun C, Li N, Guo E, et al. Overexpressed miR-9 promotes tumor metastasis via targeting E-cadherin in serous ovarian cancer. *Front Med*. 2017;11(2):214-22.
167. Sui X, Jiao YN, Yang LH, Liu J. MiR-9 accelerates epithelial-mesenchymal transition of ovarian cancer cells via inhibiting e-cadherin. *Eur Rev Med Pharmacol Sci*. 2019;23(3 Suppl):209-16.
168. Weingarten C, Jenudi Y, Tshuva RY, Moskovich D, Alfandari A, Hercbergs A, et al. The Interplay Between Epithelial-Mesenchymal Transition (EMT) and the Thyroid Hormones- α v β 3 Axis in Ovarian Cancer. *Horm Cancer*. 2018;9(1):22-32.
169. Greene J, Baird AM, Brady L, Lim M, Gray SG, McDermott R, Finn SP. Circular RNAs: Biogenesis, Function and Role in Human Diseases. *Front Mol Biosci*. 2017;4:38.
170. Cui C, Yang J, Li X, Liu D, Fu L, Wang X. Functions and mechanisms of circular RNAs in cancer radiotherapy and chemotherapy resistance. *Mol Cancer*. 2020;19(1):58.
171. Azatyan A, Zhang S, Darabi A, Siesjö P, Wang T, Zaphiropoulos PG. Circular RNAs in Hedgehog Signaling Activation and Hedgehog-Mediated Medulloblastoma Tumors. *Cancers (Basel)*. 2021;13(20).
172. Li J, Yang J, Zhou P, Le Y, Zhou C, Wang S, et al. Circular RNAs in cancer: novel insights into origins, properties, functions and implications. *Am J Cancer Res*. 2015;5(2):472-80.
173. Han YN, Xia SQ, Zhang YY, Zheng JH, Li W. Circular RNAs: A novel type of biomarker and genetic tools in cancer. *Oncotarget*. 2017;8(38):64551-63.
174. Hou LD, Zhang J. Circular RNAs: An emerging type of RNA in cancer. *Int J Immunopathol Pharmacol*. 2017;30(1):1-6.
175. Nisar S, Bhat AA, Singh M, Karedath T, Rizwan A, Hashem S, et al. Insights Into the Role of CircRNAs: Biogenesis, Characterization, Functional, and Clinical Impact in Human Malignancies. *Front Cell Dev Biol*. 2021;9:617281.
176. Tang X, Ren H, Guo M, Qian J, Yang Y, Gu C. Review on circular RNAs and new insights into their roles in cancer. *Comput Struct Biotechnol J*. 2021;19:910-28.
177. Fu L, Zhang D, Yi N, Cao Y, Wei Y, Wang W, Li L. Circular RNA circPBX3 promotes cisplatin resistance of ovarian cancer cells via interacting with IGF2BP2 to stabilize ATP7A mRNA expression. *Hum Cell*. 2022;35(5):1560-76.
178. Su M, Xiao Y, Ma J, Tang Y, Tian B, Zhang Y, et al. Circular RNAs in Cancer: emerging functions in hallmarks, stemness, resistance and roles as potential biomarkers. *Mol Cancer*. 2019;18(1):90.
179. Xu T, Wang M, Jiang L, Ma L, Wan L, Chen Q, et al. CircRNAs in anticancer drug resistance: recent advances and future potential. *Mol Cancer*. 2020;19(1):127.
180. Lim MCJ, Baird AM, Aird J, Greene J, Kapoor D, Gray SG, et al. RNAs as Candidate Diagnostic and Prognostic Markers of Prostate Cancer-From Cell Line Models to Liquid Biopsies. *Diagnostics (Basel)*. 2018;8(3).
181. Xing Y, Liang X, Lv X, Cheng Y, Du J, Liu C, Yang Y. New insights into the role of circular RNAs in ovarian cancer. *Pathol Res Pract*. 2022;238:154073.

182. Najafi S. The emerging roles and potential applications of circular RNAs in ovarian cancer: a comprehensive review. *J Cancer Res Clin Oncol*. 2022.
183. Liu M, Cao S, Guo Z, Wu Z, Meng J, Wu Y, et al. Roles and mechanisms of CircRNAs in ovarian cancer. *Front Cell Dev Biol*. 2022;10:1044897.
184. Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. *Nat Commun*. 2013;4:2126.
185. Haddad G, Lorenzen JM. Biogenesis and Function of Circular RNAs in Health and in Disease. *Front Pharmacol*. 2019;10:428.
186. Carter MG, Sharov AA, VanBuren V, Dudekula DB, Carmack CE, Nelson C, Ko MS. Transcript copy number estimation using a mouse whole-genome oligonucleotide microarray. *Genome Biol*. 2005;6(7):R61.
187. Sanger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. *Proc Natl Acad Sci U S A*. 1976;73(11):3852-6.
188. Lei M, Zheng G, Ning Q, Zheng J, Dong D. Translation and functional roles of circular RNAs in human cancer. *Mol Cancer*. 2020;19(1):30.
189. Chen LL, Yang L. Regulation of circRNA biogenesis. *RNA Biol*. 2015;12(4):381-8.
190. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. *Nature*. 2013;495(7441):384-8.
191. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*. 2013;495(7441):333-8.
192. Ma S, Kong S, Wang F, Ju S. CircRNAs: biogenesis, functions, and role in drug-resistant Tumours. *Mol Cancer*. 2020;19(1):119.
193. Xia S, Feng J, Lei L, Hu J, Xia L, Wang J, et al. Comprehensive characterization of tissue-specific circular RNAs in the human and mouse genomes. *Briefings in Bioinformatics*. 2016;18(6):984-92.
194. Mumtaz PT, Taban Q, Dar MA, Mir S, Haq ZU, Zargar SM, et al. Deep Insights in Circular RNAs: from biogenesis to therapeutics. *Biol Proced Online*. 2020;22:10.
195. Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One*. 2012;7(2):e30733.
196. Jeck WR, Sharpless NE. Detecting and characterizing circular RNAs. *Nat Biotechnol*. 2014;32(5):453-61.
197. Wang PL, Bao Y, Yee MC, Barrett SP, Hogan GJ, Olsen MN, et al. Circular RNA is expressed across the eukaryotic tree of life. *PLoS One*. 2014;9(6):e90859.
198. Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. *Nature*. 2014;505(7483):344-52.
199. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, et al. Circular RNAs are abundant, conserved, and associated with ALU repeats. *Rna*. 2013;19(2):141-57.
200. Pamudurti NR, Bartok O, Jens M, Ashwal-Fluss R, Stottmeister C, Ruhe L, et al. Translation of CircRNAs. *Mol Cell*. 2017;66(1):9-21.e7.
201. Sun P, Li G. CircCode: A Powerful Tool for Identifying circRNA Coding Ability. *Frontiers in Genetics*. 2019;10.
202. Qu S, Liu Z, Yang X, Zhou J, Yu H, Zhang R, Li H. The emerging functions and roles of circular RNAs in cancer. *Cancer Lett*. 2018;414:301-9.
203. Kong L, Xu J, Yu L, Liu S, Liu Z, Xiang J. Construction of PARPi Resistance-related Competing Endogenous RNA Network. *Curr Genomics*. 2022;23(4):262-74.
204. Teng F, Xu J, Zhang M, Liu S, Gu Y, Zhang M, et al. Comprehensive circular RNA expression profiles and the tumor-suppressive function of circHIPK3 in ovarian cancer. *Int J Biochem Cell Biol*. 2019;112:8-17.
205. Liu N, Zhang J, Zhang LY, Wang L. CircHIPK3 is upregulated and predicts a poor prognosis in epithelial ovarian cancer. *Eur Rev Med Pharmacol Sci*. 2018;22(12):3713-8.

206. Ning L, Long B, Zhang W, Yu M, Wang S, Cao D, et al. Circular RNA profiling reveals circEXOC6B and circN4BP2L2 as novel prognostic biomarkers in epithelial ovarian cancer. *Int J Oncol*. 2018;53(6):2637-46.
207. Gao Y, Zhang C, Liu Y, Wang M. Circular RNA profiling reveals circRNA1656 as a novel biomarker in high grade serous ovarian cancer. *Biosci Trends*. 2019;13(2):204-11.
208. Ge L, Sun Y, Shi Y, Liu G, Teng F, Geng Z, et al. Plasma circRNA microarray profiling identifies novel circRNA biomarkers for the diagnosis of ovarian cancer. *J Ovarian Res*. 2022;15(1):58.
209. Zhang N, Jin Y, Hu Q, Cheng S, Wang C, Yang Z, Wang Y. Circular RNA hsa_circ_0078607 suppresses ovarian cancer progression by regulating miR-518a-5p/Fas signaling pathway. *J Ovarian Res*. 2020;13(1):64.
210. Zou T, Wang PL, Gao Y, Liang WT. Circular RNA_LARP4 is lower expressed and serves as a potential biomarker of ovarian cancer prognosis. *Eur Rev Med Pharmacol Sci*. 2018;22(21):7178-82.
211. Luo Y, Gui R. Circulating exosomal circFoxp1 confers cisplatin resistance in epithelial ovarian cancer cells. *J Gynecol Oncol*. 2020;31(5):e75.
212. Li H, Luo F, Jiang X, Zhang W, Xiang T, Pan Q, et al. CircITGB6 promotes ovarian cancer cisplatin resistance by resetting tumor-associated macrophage polarization toward the M2 phenotype. *J Immunother Cancer*. 2022;10(3).
213. Chen Q, Zhang J, He Y, Wang Y. hsa_circ_0061140 Knockdown Reverses FOXM1-Mediated Cell Growth and Metastasis in Ovarian Cancer through miR-370 Sponge Activity. *Mol Ther Nucleic Acids*. 2018;13:55-63.
214. Liu X, Yin Z, Wu Y, Zhan Q, Huang H, Fan J. Circular RNA lysophosphatidic acid receptor 3 (circ-LPAR3) enhances the cisplatin resistance of ovarian cancer. *Bioengineered*. 2022;13(2):3739-50.
215. Zhang L, Zhou Q, Qiu Q, Hou L, Wu M, Li J, et al. CircPLEKHM3 acts as a tumor suppressor through regulation of the miR-9/BRCA1/DNAJB6/KLF4/AKT1 axis in ovarian cancer. *Mol Cancer*. 2019;18(1):144.
216. Zhang S, Cheng J, Quan C, Wen H, Feng Z, Hu Q, et al. circCELSR1 (hsa_circ_0063809) Contributes to Paclitaxel Resistance of Ovarian Cancer Cells by Regulating FOXR2 Expression via miR-1252. *Mol Ther Nucleic Acids*. 2020;19:718-30.
217. Sheng H, Pan H, Yao M, Xu L, Lu J, Liu B, et al. Integrated Analysis of Circular RNA-Associated ceRNA Network Reveals Potential circRNA Biomarkers in Human Breast Cancer. *Comput Math Methods Med*. 2021;2021:1732176.
218. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature*. 2005;434(7035):913-7.
219. Singh D, Kesharwani P, Alhakamy NA, Siddique HR. Accentuating CircRNA-miRNA-Transcription Factors Axis: A Conundrum in Cancer Research. *Frontiers in Pharmacology*. 2022;12.
220. Sakshi S, Jayasuriya R, Ganesan K, Xu B, Ramkumar KM. Role of circRNA-miRNA-mRNA interaction network in diabetes and its associated complications. *Mol Ther Nucleic Acids*. 2021;26:1291-302.
221. Frisk NLS, Sørensen AE, Pedersen OBV, Dalgaard LT. Circulating microRNAs for Early Diagnosis of Ovarian Cancer: A Systematic Review and Meta-Analysis. *Biomolecules*. 2023;13(5).
222. Panda AC. Circular RNAs Act as miRNA Sponges. *Adv Exp Med Biol*. 2018;1087:67-79.
223. Guo Z, Cao Q, Zhao Z, Song C. Biogenesis, Features, Functions, and Disease Relationships of a Specific Circular RNA: CDR1as. *Aging Dis*. 2020;11(4):1009-20.
224. Sui W, Shi Z, Xue W, Ou M, Zhu Y, Chen J, et al. Circular RNA and gene expression profiles in gastric cancer based on microarray chip technology. *Oncol Rep*. 2017;37(3):1804-14.
225. Hanker LC, Loibl S, Burchardi N, Pfisterer J, Meier W, Pujade-Lauraine E, et al. The impact of second to sixth line therapy on survival of relapsed ovarian cancer after primary taxane/platinum-based therapy. *Ann Oncol*. 2012;23(10):2605-12.
226. Ortiz M, Wabel E, Mitchell K, Horibata S. Mechanisms of chemotherapy resistance in ovarian cancer. *Cancer Drug Resist*. 2022;5(2):304-16.

227. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. 2005;434(7035):917-21.
228. Alemzadeh E, Allahqoli L, Mazidimoradi A, Alemzadeh E, Ghasemi F, Salehiniya H, Alkatout I. Deciphering resistance mechanisms and novel strategies to overcome drug resistance in ovarian cancer: a comprehensive review. *Oncol Res*. 2024;32(5):831-47.
229. Kalayda GV, Wagner CH, Jaehde U. Relevance of copper transporter 1 for cisplatin resistance in human ovarian carcinoma cells. *J Inorg Biochem*. 2012;116:1-10.
230. Li T, Peng J, Zeng F, Zhang K, Liu J, Li X, et al. Association between polymorphisms in CTR1, CTR2, ATP7A, and ATP7B and platinum resistance in epithelial ovarian cancer. *Int J Clin Pharmacol Ther*. 2017;55(10):774-80.
231. Mangala LS, Zuzel V, Schmandt R, Leshane ES, Halder JB, Armaiz-Pena GN, et al. Therapeutic Targeting of ATP7B in Ovarian Carcinoma. *Clin Cancer Res*. 2009;15(11):3770-80.
232. Du P, Wang Y, Chen L, Gan Y, Wu Q. High ERCC1 expression is associated with platinum-resistance, but not survival in patients with epithelial ovarian cancer. *Oncol Lett*. 2016;12(2):857-62.
233. Maloney SM, Hoover CA, Morejon-Lasso LV, Prosperi JR. Mechanisms of Taxane Resistance. *Cancers (Basel)*. 2020;12(11).
234. Orr GA, Verdier-Pinard P, McDaid H, Horwitz SB. Mechanisms of Taxol resistance related to microtubules. *Oncogene*. 2003;22(47):7280-95.
235. Vahedi S, Chufan EE, Ambudkar SV. Global alteration of the drug-binding pocket of human P-glycoprotein (ABCB1) by substitution of fifteen conserved residues reveals a negative correlation between substrate size and transport efficiency. *Biochem Pharmacol*. 2017;143:53-64.
236. van Eijk M, Boosman RJ, Schinkel AH, Huitema ADR, Beijnen JH. Cytochrome P450 3A4, 3A5, and 2C8 expression in breast, prostate, lung, endometrial, and ovarian tumors: relevance for resistance to taxanes. *Cancer Chemother Pharmacol*. 2019;84(3):487-99.
237. Whitaker RH, Placzek WJ. Regulating the BCL2 Family to Improve Sensitivity to Microtubule Targeting Agents. *Cells*. 2019;8(4).
238. Ben-Hamo R, Zilberberg A, Cohen H, Bahar-Shany K, Wachtel C, Korach J, et al. Resistance to paclitaxel is associated with a variant of the gene BCL2 in multiple tumor types. *NPJ Precis Oncol*. 2019;3:12.
239. Dias MP, Moser SC, Ganesan S, Jonkers J. Understanding and overcoming resistance to PARP inhibitors in cancer therapy. *Nat Rev Clin Oncol*. 2021;18(12):773-91.
240. D'Andrea AD. Mechanisms of PARP inhibitor sensitivity and resistance. *DNA Repair (Amst)*. 2018;71:172-6.
241. Cordani N, Bianchi T, Ammoni LC, Cortinovis DL, Cazzaniga ME, Lissoni AA, et al. An Overview of PARP Resistance in Ovarian Cancer from a Molecular and Clinical Perspective. *International Journal of Molecular Sciences*. 2023;24(15):11890.
242. Kondrashova O, Nguyen M, Shield-Artin K, Tinker AV, Teng NNH, Harrell MI, et al. Secondary Somatic Mutations Restoring RAD51C and RAD51D Associated with Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma. *Cancer Discov*. 2017;7(9):984-98.
243. Lin KK, Harrell MI, Oza AM, Oaknin A, Ray-Coquard I, Tinker AV, et al. BRCA Reversion Mutations in Circulating Tumor DNA Predict Primary and Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma. *Cancer Discov*. 2019;9(2):210-9.
244. Edwards SL, Brough R, Lord CJ, Natrajan R, Vatcheva R, Levine DA, et al. Resistance to therapy caused by intragenic deletion in BRCA2. *Nature*. 2008;451(7182):1111-5.
245. Collet L, Hanvic B, Turinetto M, Treilleux I, Chopin N, Le Saux O, Ray-Coquard I. BRCA1/2 alterations and reversion mutations in the area of PARP inhibitors in high grade ovarian cancer: state of the art and forthcoming challenges. *Front Oncol*. 2024;14:1354427.
246. ESMO. ESMO E-Learning: Management of BRCA-Mutated Ovarian Cancer - An Update. In: Ledermann J, editor. 2024.
247. Chu YY, Yam C, Yamaguchi H, Hung MC. Biomarkers beyond BRCA: promising combinatorial treatment strategies in overcoming resistance to PARP inhibitors. *J Biomed Sci*. 2022;29(1):86.

248. Ray Chaudhuri A, Callen E, Ding X, Gogola E, Duarte AA, Lee JE, et al. Replication fork stability confers chemoresistance in BRCA-deficient cells. *Nature*. 2016;535(7612):382-7.
249. Wu Y, Xu S, Cheng S, Yang J, Wang Y. Clinical application of PARP inhibitors in ovarian cancer: from molecular mechanisms to the current status. *J Ovarian Res*. 2023;16(1):6.
250. Pettitt SJ, Krastev DB, Brandsma I, Dréan A, Song F, Aleksandrov R, et al. Genome-wide and high-density CRISPR-Cas9 screens identify point mutations in PARP1 causing PARP inhibitor resistance. *Nature Communications*. 2018;9(1):1849.
251. Pina PS, Lheureux S. Overcoming PARP inhibitor resistance in ovarian cancer. *International Journal of Gynecologic Cancer*. 2023;33(3):364-76.
252. Tan J, Zheng B, Zhou S. Deciphering the "Rosetta Stone" of ovarian cancer stem cells: Opportunities and challenges. *Biochim Biophys Acta Rev Cancer*. 2025;1880(3):189346.
253. Xiao H, Cheng G, Zhang H, Liu Y, Chen Z, Gao Y, et al. Role of KLF5 in enhancing ovarian cancer stemness and PARPi resistance: mechanisms and therapeutic targeting. *J Transl Med*. 2025;23(1):492.
254. Wu Y, Wang T, Xia L, Zhang M. LncRNA WDFY3-AS2 promotes cisplatin resistance and the cancer stem cell in ovarian cancer by regulating hsa-miR-139-5p/SDC4 axis. *Cancer Cell Int*. 2021;21(1):284.
255. Neveling K, Mensenkamp AR, Derks R, Kwint M, Ouchene H, Steehouwer M, et al. BRCA Testing by Single-Molecule Molecular Inversion Probes. *Clin Chem*. 2017;63(2):503-12.
256. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-24.
257. KMPlot: A Web-Based Tool for Prognostic Gene Expression Analysis in Cancer. [Internet]. 2009 [cited 22/02/2024]. Available from: <https://kmplot.com/analysis/index.php?p=service>.
258. Agarwal V, Bell GW, Nam J-W, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *eLife*. 2015;4:e05005.
259. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2(5):401-4.
260. de Bruijn I, Kundra R, Mastrogiacomio B, Tran TN, Sikina L, Mazor T, et al. Analysis and Visualization of Longitudinal Genomic and Clinical Data from the AACR Project GENIE Biopharma Collaborative in cBioPortal. *Cancer Res*. 2023;83(23):3861-7.
261. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6(269):pl1.
262. DSMZ. Cell Line Data 2023 [Available from: <https://www.dsmz.de/collection/digital-collection/cell-line-data>]
263. Bairoch A. Cellosaurus 2023 [cited 2023 19/09/2023]. Available from: <https://www.cellosaurus.org/>.
264. Boyle EA, O'Roak BJ, Martin BK, Kumar A, Shendure J. MIPgen: optimized modeling and design of molecular inversion probes for targeted resequencing. *Bioinformatics*. 2014;30(18):2670-2.
265. systems Jm. SEQUENCE Pilot: **JSI medical systems GmbH, Tullastrasse 18, 77955 Ettenheim, Germany**; 2025 [Available from: <https://www.jsi-medisys.de/products/sequence-pilot/>].
266. D'Achille G, Morroni G. Side effects of antibiotics and perturbations of mitochondria functions. *Int Rev Cell Mol Biol*. 2023;377:121-39.
267. Llobet L, Montoya J, López-Gallardo E, Ruiz-Pesini E. Side Effects of Culture Media Antibiotics on Cell Differentiation. *Tissue Eng Part C Methods*. 2015;21(11):1143-7.
268. Drexler HG, Uphoff CC. Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. *Cytotechnology*. 2002;39(2):75-90.
269. Young L, Sung J, Stacey G, Masters JR. Detection of Mycoplasma in cell cultures. *Nature Protocols*. 2010;5(5):929-34.

270. McDermott M, Eustace AJ, Busschots S, Breen L, Crown J, Clynes M, et al. In vitro Development of Chemotherapy and Targeted Therapy Drug-Resistant Cancer Cell Lines: A Practical Guide with Case Studies. *Front Oncol.* 2014;4:40.
271. NCCP. NCCP National SACT Regimens: 2023 Health Service Executive; 2023 [Available from: <https://www.hse.ie/eng/services/list/5/cancer/profinfo/chemoprotocols/gynaecology/>].
272. Oguri S, Sakakibara T, Mase H, Shimizu T, Ishikawa K, Kimura K, Smyth RD. Clinical pharmacokinetics of carboplatin. *J Clin Pharmacol.* 1988;28(3):208-15.
273. Selleck. Carboplatin Data Sheet 2023 [cited 2023. Available from: <https://www.selleckchem.com/datasheet/Carboplatin-S121510-DataSheet.html>].
274. Mross K, Holländer N, Hauns B, Schumacher M, Maier-Lenz H. The pharmacokinetics of a 1-h paclitaxel infusion. *Cancer Chemother Pharmacol.* 2000;45(6):463-70.
275. Selleck. Paclitaxel Datasheet: Selleck; 2023 [Available from: [https://www.selleckchem.com/datasheet/Paclitaxel\(Taxol\)-S115012-DataSheet.html](https://www.selleckchem.com/datasheet/Paclitaxel(Taxol)-S115012-DataSheet.html)].
276. Rolfo C, de Vos-Geelen J, Isambert N, Molife LR, Schellens JHM, De Grève J, et al. Pharmacokinetics and Safety of Olaparib in Patients with Advanced Solid Tumours and Renal Impairment. *Clin Pharmacokinet.* 2019;58(9):1165-74.
277. Pohl G, Ho CL, Kurman RJ, Bristow R, Wang TL, Shih Ie M. Inactivation of the mitogen-activated protein kinase pathway as a potential target-based therapy in ovarian serous tumors with KRAS or BRAF mutations. *Cancer Res.* 2005;65(5):1994-2000.
278. Yamamoto N, Nokihara H, Yamada Y, Goto Y, Tanioka M, Shibata T, et al. A Phase I, dose-finding and pharmacokinetic study of olaparib (AZD2281) in Japanese patients with advanced solid tumors. *Cancer Sci.* 2012;103(3):504-9.
279. Selleck. Olaparib Datasheet: Selleck; 2023 [Available from: [https://www.selleckchem.com/datasheet/AZD2281\(Olaparib\)-S106024-DataSheet.html](https://www.selleckchem.com/datasheet/AZD2281(Olaparib)-S106024-DataSheet.html)].
280. Laird PW, Zijderfeld A, Linders K, Rudnicki MA, Jaenisch R, Berns A. Simplified mammalian DNA isolation procedure. *Nucleic Acids Res.* 1991;19(15):4293.
281. Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS. MicroRNA targets in *Drosophila*. *Genome Biol.* 2003;5(1):R1.
282. Pasquinelli AE. MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. *Nat Rev Genet.* 2012;13(4):271-82.
283. Kotsopoulos J, Gronwald J, Karlan B, Rosen B, Huzarski T, Moller P, et al. Age-specific ovarian cancer risks among women with a BRCA1 or BRCA2 mutation. *Gynecol Oncol.* 2018;150(1):85-91.
284. Surveillance E, End Result Cancer Stat Facts: Ovarian Cancer 2019 [Available from: <https://seer.cancer.gov/statfacts/html/ovary.html>].
285. Takamatsu S, Yoshihara K, Baba T, Shimada M, Yoshida H, Kajiyama H, et al. Prognostic relevance of HRDness gene expression signature in ovarian high-grade serous carcinoma; JGOG3025-TR2 study. *Br J Cancer.* 2023.
286. Konstantinopoulos PA, Norquist B, Lacchetti C, Armstrong D, Grisham RN, Goodfellow PJ, et al. Germline and Somatic Tumor Testing in Epithelial Ovarian Cancer: ASCO Guideline. *Journal of Clinical Oncology.* 2020;38(11):1222-45.
287. (NCCP) NCCP. GUIDANCE FOR MEDICAL ONCOLOGISTS ON TESTING REQUIREMENTS BY DIRECT ORDERING TO INFORM PARPI TREATMENT OPTIONS 2023 [updated 29/11/2023; cited 2024 09 MAY 2024]. Available from: <https://www.hse.ie/eng/services/list/5/cancer/profinfo/medonc/sactguidance/guidance-for-medical-oncologists-on-testing-requirements-by-direct-ordering-to-inform-parpi-treatment-options.pdf>.
288. Sia TY, Maio A, Kemel YM, Arora KS, Gordhandas SB, Kahn RM, et al. Germline Pathogenic Variants and Genetic Counseling by Ancestry in Patients With Epithelial Ovarian Cancer. *JCO Precis Oncol.* 2023;7:e2300137.

289. Figlioli G, De Nicolo A, Catucci I, Manoukian S, Peissel B, Azzollini J, et al. Analysis of Italian BRCA1/2 Pathogenic Variants Identifies a Private Spectrum in the Population from the Bergamo Province in Northern Italy. *Cancers (Basel)*. 2021;13(3).
290. Kowalik A, Siołek M, Kopczyński J, Krawiec K, Kalisz J, Zięba S, et al. BRCA1 founder mutations and beyond in the Polish population: A single-institution BRCA1/2 next-generation sequencing study. *PLoS one*. 2018;13(7):e0201086-e.
291. Ferla R, Calò V, Cascio S, Rinaldi G, Badalamenti G, Carreca I, et al. Founder mutations in BRCA1 and BRCA2 genes. *Ann Oncol*. 2007;18 Suppl 6:vi93-8.
292. Helpman L, Zidan O, Friedman E, Kalfon S, Perri T, Ben-Baruch G, Korach J. Young Israeli women with epithelial ovarian cancer: prevalence of BRCA mutations and clinical correlates. *J Gynecol Oncol*. 2017;28(5):e61.
293. de Freitas Ribeiro AA, Junior NMC, Dos Santos LL. Systematic review of the molecular basis of hereditary breast and ovarian cancer syndrome in Brazil: the current scenario. *Eur J Med Res*. 2024;29(1):187.
294. Wu Z, Zhang Q, Jin Y, Zhang X, Chen Y, Yang C, et al. Population-based BRCA germline mutation screening in the Han Chinese identifies individuals at risk of BRCA mutation-related cancer: experience from a clinical diagnostic center from greater Shanghai area. *BMC Cancer*. 2024;24(1):411.
295. Kalachand RD, O'Riain C, Toomey S, Carr A, Timms KM, O'Toole S, et al. Prevalence of tumor BRCA1 and BRCA2 dysfunction in unselected patients with ovarian cancer. *Obstet Gynecol Sci*. 2020;63(5):643-54.
296. Minig L, Gracia Segovia M, Arencibia O, Zorrero C, Marti L, García Pineda V, et al. Oncological outcomes among young women with non-epithelial ovarian cancer: the YOC-Care study (Young Ovarian Cancer – Care). *International Journal of Gynecological Cancer*. 2023;33(6):915-21.
297. Institute NC. Cancer stat facts: ovarian cancer. [internet publication]. 2022.
298. BEHTASH N, GHAYOURI AZAR E, FAKHREJAHANI F. Symptoms of ovarian cancer in young patients 2 years before diagnosis, a case–control study. *European Journal of Cancer Care*. 2008;17(5):483-7.
299. Kim SY, Lee JR. Fertility preservation option in young women with ovarian cancer. *Future Oncol*. 2016;12(14):1695-8.
300. Zhou X, Edmonson MN, Wilkinson MR, Patel A, Wu G, Liu Y, et al. Exploring genomic alteration in pediatric cancer using ProteinPaint. *Nat Genet*. 2016;48(1):4-6.
301. Morgan RD, Burghel GJ, Flaum N, Bulman M, Smith P, Clamp AR, et al. BRCA1/2 in non-mucinous epithelial ovarian cancer: tumour with or without germline testing? *Br J Cancer*. 2022;127(1):163-7.
302. Shao C, Wan J, Lam FC, Tang H, Marley AR, Song Y, et al. A comprehensive literature review and meta-analysis of the prevalence of pan-cancer BRCA mutations, homologous recombination repair gene mutations, and homologous recombination deficiencies. *Environ Mol Mutagen*. 2022;63(6):308-16.
303. Flaum N, Morgan RD, Burghel GJ, Bulman M, Clamp AR, Hasan J, et al. Mainstreaming germline BRCA1/2 testing in non-mucinous epithelial ovarian cancer in the North West of England. *Eur J Hum Genet*. 2020;28(11):1541-7.
304. Pal T, Permuth-Wey J, Betts JA, Krischer JP, Fiorica J, Arango H, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. *Cancer*. 2005;104(12):2807-16.
305. Risch HA, McLaughlin JR, Cole DE, Rosen B, Bradley L, Fan I, et al. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. *J Natl Cancer Inst*. 2006;98(23):1694-706.
306. Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, et al. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. *J Clin Oncol*. 2012;30(21):2654-63.

307. Bu H, Chen J, Li Q, Hou J, Wei Y, Yang X, et al. BRCA mutation frequency and clinical features of ovarian cancer patients: A report from a Chinese study group. *J Obstet Gynaecol Res*. 2019;45(11):2267-74.
308. Karami F, Mehdipour P. A comprehensive focus on global spectrum of BRCA1 and BRCA2 mutations in breast cancer. *Biomed Res Int*. 2013;2013:928562.
309. Whittemore AS, Gong G, John EM, McGuire V, Li FP, Ostrow KL, et al. Prevalence of BRCA1 mutation carriers among U.S. non-Hispanic Whites. *Cancer Epidemiol Biomarkers Prev*. 2004;13(12):2078-83.
310. Borg A, Haile RW, Malone KE, Capanu M, Diep A, Törngren T, et al. Characterization of BRCA1 and BRCA2 deleterious mutations and variants of unknown clinical significance in unilateral and bilateral breast cancer: the WECARE study. *Hum Mutat*. 2010;31(3):E1200-40.
311. Neuhausen SL, Mazoyer S, Friedman L, Stratton M, Offit K, Caligo A, et al. Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. *Am J Hum Genet*. 1996;58(2):271-80.
312. Liede A, Cohen B, Black DM, Davidson RH, Renwick A, Hoodfar E, et al. Evidence of a founder BRCA1 mutation in Scotland. *Br J Cancer*. 2000;82(3):705-11.
313. ClinVar NCFBI. VCV000051640.45 [7/7/2025]. Available from: <https://www.ncbi.nlm.nih.gov/clinvar/variation/51640/#new-germline-citation>.
314. Tayefi M, Ngo P, Chomutare T, Dalianis H, Salvi E, Budrionis A, Godtliobsen F. Challenges and opportunities beyond structured data in analysis of electronic health records. *WIREs Computational Statistics*. 2021;13(6):e1549.
315. Vergote I, González-Martín A, Ray-Coquard I, Harter P, Colombo N, Pujol P, et al. European experts consensus: BRCA/homologous recombination deficiency testing in first-line ovarian cancer. *Ann Oncol*. 2022;33(3):276-87.
316. Care M, McCuaig J, Clarke B, Grenier S, Kim RH, Rouzbahman M, et al. Tumor and germline next generation sequencing in high grade serous cancer: experience from a large population-based testing program. *Mol Oncol*. 2021;15(1):80-90.
317. Ran X, Jing H, Li Z. The clinical features and management of Lynch syndrome-associated ovarian cancer. *Journal of Obstetrics and Gynaecology Research*. 2022;48(7):1538-45.
318. Cercek A, Lumish M, Sinopoli J, Weiss J, Shia J, Lamendola-Essel M, et al. PD-1 Blockade in Mismatch Repair–Deficient, Locally Advanced Rectal Cancer. *New England Journal of Medicine*. 2022;386(25):2363-76.
319. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357(6349):409-13.
320. Mirza MR, Chase DM, Slomovitz BM, dePont Christensen R, Novák Z, Black D, et al. Dostarlimab for Primary Advanced or Recurrent Endometrial Cancer. *N Engl J Med*. 2023;388(23):2145-58.
321. Kristeleit R, Devlin MJ, Clamp A, Gourley C, Roux R, Hall M, et al. Pembrolizumab in Patients With Advanced Clear Cell Gynecological Cancer: A Phase 2 Nonrandomized Clinical Trial. *JAMA Oncol*. 2025;11(4):377-85.
322. Friedman CF, Manning-Geist BL, Zhou Q, Soumerai T, Holland A, Da Cruz Paula A, et al. Nivolumab for mismatch-repair-deficient or hypermutated gynecologic cancers: a phase 2 trial with biomarker analyses. *Nat Med*. 2024;30(5):1330-8.
323. Ngoi NYL, Heong V, Ow S, Chay WY, Kim HS, Choi CH, et al. A multicenter phase II randomized trial of durvalumab (MEDI-4736) versus physician's choice chemotherapy in recurrent ovarian clear cell adenocarcinoma (MOCCA). *International Journal of Gynecological Cancer*. 2020;30(8):1239-42.
324. Flaum N, Crosbie EJ, Woodward ER, Laloo F, Morgan R, Ryan N, Evans DG. MSH2 is the very young onset ovarian cancer predisposition gene, not BRCA1. *J Med Genet*. 2023;60(6):576-7.
325. Haggstrom LR, Lee YC, Scott CL, Ledermann JA, Gourley C, McNeish I, et al. 764P How long is long enough? An international survey exploring practice variations on the recommended duration of

maintenance therapy with PARP inhibitors in platinum sensitive recurrent ovarian cancer and long-term outcomes. *Annals of Oncology*. 2024;35:S578-S9.

326. Margalit O, Stemmer A, Chapin WJ, Shacham-Shmueli E, Kopetz S, Andre T, et al. Duration of immunotherapy in dMMR/MSI-H metastatic colorectal cancer patients. *European Journal of Cancer*. 2024;212:114336.

327. Su HI, Lacchetti C, Letourneau J, Partridge AH, Qamar R, Quinn GP, et al. Fertility Preservation in People With Cancer: ASCO Guideline Update. *Journal of Clinical Oncology*. 2025;43(12):1488-515.

328. Commission E. Ireland 2024 Digital Decade Country Report. 2024.

329. Howlett E. Electronic health records and citizen access to health information. 2024.

330. Loomans-Kropp HA, Umar A. Cancer prevention and screening: the next step in the era of precision medicine. *NPJ Precis Oncol*. 2019;3:3.

331. Ireland HRC. Unlocking the Potential of Patient Registries: A Guide for Success. 2023.

332. Ireland HRC. Is it time to get serious about patient registries? 2025 [Available from: <https://hrci.ie/may-2025-is-it-time-to-get-serious-about-patient-registries/#:~:text=This%20video%20was%20created%20for,and%20support%20better%20decision%20making>].

333. Jackson AD, Goss CH. Epidemiology of CF: How registries can be used to advance our understanding of the CF population. *J Cyst Fibros*. 2018;17(3):297-305.

334. Dublin TC. The RITA-Ireland (RIV) Registry and Biobank 2024 [Available from: <https://www.tcd.ie/medicine/thkc/our-research/rita-ireland-vasculitis-riv-registry-and-biobank/>].

335. Butler E, Collier S, Boland M, Hanhauser Y, Connolly E, Hevey D. Self-concept and health anxiety relate to psychological outcomes for BRCA1/2 carriers. *Psychooncology*. 2020;29(10):1638-45.

336. Butler E, Collier S, Hevey D. The factors associated with distress a minimum of six months after BRCA1/2 confirmation: A systematic review. *J Psychosoc Oncol*. 2021;39(5):646-72.

337. Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation) (Text with EEA relevance), (2016).

338. S.I. No. 18/2021 - Data Protection Act 2018 (Section 36(2)) (Health Research) (Amendment) Regulations 2021, (2018).

339. Hospital SJ. Data Protection Office 2025 [Available from: <https://www.stjames.ie/services/corporateaffairs/dataprotectionoffice/>].

340. England N. National Inherited Cancer Predisposition Register: NHS England; 2025 [Available from: <https://digital.nhs.uk/ndrs/our-work/genomics/nicpr>].

341. Colomer-Lahiguera S, Steimer M, Ellis U, Eicher M, Tompson M, Corbière T, Haase KR. Patient and public involvement in cancer research: A scoping review. *Cancer Med*. 2023;12(14):15530-43.

342. Boote J, Wong R, Booth A. 'Talking the talk or walking the walk?' A bibliometric review of the literature on public involvement in health research published between 1995 and 2009. *Health Expect*. 2015;18(1):44-57.

343. Rahman A, Nawaz S, Khan E, Islam S. Nothing about us, without us: is for us. *Research Involvement and Engagement*. 2022;8(1):39.

344. gov.ie. Minister for Health publishes Bill to provide a legal basis for digital health records in Ireland. In: Health Do, editor. 22 July 20242024.

345. Group UCG. NDRS NICPR 2025 [Available from: <https://www.ukcgg.org/information-education/ndrs-nicpr/>].

346. Vasen HF, Velthuisen ME, Kleibeuker JH, Menko FH, Nagengast FM, Cats A, et al. Hereditary cancer registries improve the care of patients with a genetic predisposition to cancer: contributions from the Dutch Lynch syndrome registry. *Fam Cancer*. 2016;15(3):429-35.

347. Ljubljana IoO. Report of the national Registry of Tested Individuals from Families with Hereditary Cancer for the Period. 2025.
348. Ireland CFR. 20 Years and Counting, Anniversary Report. 2023.
349. Beg A, Parveen R, Fouad H, Yahia ME, Hassanein AS. Identification of Driver Genes and miRNAs in Ovarian Cancer through an Integrated In-Silico Approach. *Biology (Basel)*. 2023;12(2).
350. Hwang H, Jeon H, Yeo N, Baek D. Big data and deep learning for RNA biology. *Experimental & Molecular Medicine*. 2024;56(6):1293-321.
351. Marques L, Costa B, Pereira M, Silva A, Santos J, Saldanha L, et al. Advancing Precision Medicine: A Review of Innovative In Silico Approaches for Drug Development, Clinical Pharmacology and Personalized Healthcare. *Pharmaceutics*. 2024;16(3):332.
352. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol*. 2008;10(5):593-601.
353. Condrat CE, Thompson DC, Barbu MG, Bugnar OL, Boboc A, Cretoiu D, et al. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. *Cells*. 2020;9(2).
354. Davies M, Davey MG, Miller N. The Potential of MicroRNAs as Clinical Biomarkers to Aid Ovarian Cancer Diagnosis and Treatment. *Genes (Basel)*. 2022;13(11).
355. Zhang Z, Chang M, Song X, Wang K, Sun W, Ma H, et al. TIPE1 Suppresses Growth and Metastasis of Ovarian Cancer. *J Oncol*. 2021;2021:5538911.
356. Gao H, Zhang Z, Jiang L, Zhang L, Qin L, Liu T, Yang S. TNFAIP8 variants as potential epidemiological and predictive biomarkers in ovarian cancer. *Cancer Cell Int*. 2020;20:396.
357. Xie Y, Zhou F, Zhao X. TNFAIP8 promotes cell growth by regulating the Hippo pathway in epithelial ovarian cancer. *Exp Ther Med*. 2018;16(6):4975-82.
358. Liu T, Xia B, Lu Y, Xu Y, Lou G. TNFAIP8 overexpression is associated with platinum resistance in epithelial ovarian cancers with optimal cytoreduction. *Hum Pathol*. 2014;45(6):1251-7.
359. Liu T, Gao H, Chen X, Lou G, Gu L, Yang M, et al. TNFAIP8 as a predictor of metastasis and a novel prognostic biomarker in patients with epithelial ovarian cancer. *Br J Cancer*. 2013;109(6):1685-92.
360. Wang J, Gao H, Liu G, Gu L, Yang C, Zhang F, Liu T. Tumor necrosis factor α -induced protein 8 expression as a predictor of prognosis and resistance in patients with advanced ovarian cancer treated with neoadjuvant chemotherapy. *Hum Pathol*. 2018;82:239-48.
361. Qiao HY, Zhang Q, Wang JM, Jiang JY, Huan LY, Yan J, et al. TRIM29 regulates the SETBP1/SET/PP2A axis via transcription factor VEZF1 to promote progression of ovarian cancer. *Cancer Lett*. 2022;529:85-99.
362. Zhu M, Ye C, Wang J, Yang G, Ying X. Activation of COL11A1 by PRRX1 promotes tumor progression and radioresistance in ovarian cancer. *Int J Radiat Biol*. 2021;97(7):958-67.
363. Zuo L, Li X, Tan Y, Zhu H, Xiao M. Prospective pathway signaling and prognostic values of MicroRNA-9 in ovarian cancer based on gene expression omnibus (GEO): a bioinformatics analysis. *J Ovarian Res*. 2021;14(1):29.
364. Song IH, Kim KR, Lim S, Kim SH, Sung CO. Expression and prognostic significance of epithelial-mesenchymal transition-related markers and phenotype in serous ovarian cancer. *Pathol Res Pract*. 2018;214(10):1564-71.
365. Dufresne J, Bowden P, Thavarajah T, Florentinus-Mefailoski A, Chen ZZ, Tucholska M, et al. The plasma peptides of ovarian cancer. *Clin Proteomics*. 2018;15:41.
366. Chen W, Ru J, Wu T, Man D, Wu J, Wu L, et al. MiR-652-3p promotes malignancy and metastasis of cancer cells via inhibiting TNRC6A in hepatocellular carcinoma. *Biochem Biophys Res Commun*. 2023;640:1-11.
367. Li M, Zhai P, Mu X, Song J, Zhang H, Su J. Hypoxic BMSC-derived exosomal miR-652-3p promotes proliferation and metastasis of hepatocarcinoma cancer cells via targeting TNRC6A. *Aging (Albany NY)*. 2023;15(22):12780-93.

368. Chi X, Jiang Y, Chen Y, Lv L, Chen J, Yang F, et al. Upregulation of microRNA miR-652-3p is a prognostic risk factor for hepatocellular carcinoma and regulates cell proliferation, migration, and invasion. *Bioengineered*. 2021;12(1):7519-28.
369. Zhu QL, Zhan DM, Chong YK, Ding L, Yang YG. MiR-652-3p promotes bladder cancer migration and invasion by targeting KCNN3. *Eur Rev Med Pharmacol Sci*. 2019;23(20):8806-12.
370. Nam RK, Benatar T, Amemiya Y, Wallis CJD, Romero JM, Tsagaris M, et al. MicroRNA-652 induces NED in LNCaP and EMT in PC3 prostate cancer cells. *Oncotarget*. 2018;9(27):19159-76.
371. Ji D, Qiao M, Yao Y, Li M, Chen H, Dong Q, et al. Serum-based microRNA signature predicts relapse and therapeutic outcome of adjuvant chemotherapy in colorectal cancer patients. *EBioMedicine*. 2018;35:189-97.
372. Mangolini A, Ferracin M, Zanzi MV, Saccenti E, Ebnaof SO, Poma VV, et al. Diagnostic and prognostic microRNAs in the serum of breast cancer patients measured by droplet digital PCR. *Biomark Res*. 2015;3:12.
373. Yang W, Zhou C, Luo M, Shi X, Li Y, Sun Z, et al. MiR-652-3p is upregulated in non-small cell lung cancer and promotes proliferation and metastasis by directly targeting Lgl1. *Oncotarget*. 2016;7(13):16703-15.
374. Chang S, Sharan SK. BRCA1 and microRNAs: emerging networks and potential therapeutic targets. *Mol Cells*. 2012;34(5):425-32.
375. Wu RL, Ali S, Bandyopadhyay S, Alosch B, Hayek K, Daaboul MF, et al. Comparative Analysis of Differentially Expressed miRNAs and their Downstream mRNAs in Ovarian Cancer and its Associated Endometriosis. *J Cancer Sci Ther*. 2015;7(7):258-65.
376. Chong GO, Jeon HS, Han HS, Son JW, Lee YH, Hong DG, et al. Differential MicroRNA Expression Profiles in Primary and Recurrent Epithelial Ovarian Cancer. *Anticancer Res*. 2015;35(5):2611-7.
377. Dong L, Cao X, Luo Y, Zhang G, Zhang D. A Positive Feedback Loop of lncRNA DSCR8/miR-98-5p/STAT3/HIF-1 α Plays a Role in the Progression of Ovarian Cancer. *Front Oncol*. 2020;10:1713.
378. Wang Y, Bao W, Liu Y, Wang S, Xu S, Li X, et al. miR-98-5p contributes to cisplatin resistance in epithelial ovarian cancer by suppressing miR-152 biogenesis via targeting Dicer1. *Cell Death Dis*. 2018;9(5):447.
379. Guo H, Ha C, Dong H, Yang Z, Ma Y, Ding Y. Cancer-associated fibroblast-derived exosomal microRNA-98-5p promotes cisplatin resistance in ovarian cancer by targeting CDKN1A. *Cancer Cell Int*. 2019;19:347.
380. Qi X, Yu C, Wang Y, Lin Y, Shen B. Network vulnerability-based and knowledge-guided identification of microRNA biomarkers indicating platinum resistance in high-grade serous ovarian cancer. *Clin Transl Med*. 2019;8(1):28.
381. Zhang YQ, Shi SS, Li YF, Yang Y, Bai P, Qiao CH. Melatonin Inhibits Gastric Cancer Cell Proliferation by Suppressing Exosome miR-27b-3p Expression. *Anticancer Res*. 2023;43(10):4413-24.
382. Yang X, Chen J, Liao Y, Huang L, Wen C, Lin M, et al. MiR-27b-3p promotes migration and invasion in colorectal cancer cells by targeting HOXA10. *Biosci Rep*. 2019;39(12).
383. Yang L, Ruan Y, Chen B, Zhu Y, Xu H. Circ_0001671 regulates prostate cancer progression through miR-27b-3p/BLM axis. *Sci Rep*. 2024;14(1):12181.
384. Xia M, Duan LJ, Lu BN, Pang YZ, Pang ZR. LncRNA AFAP1-AS1/miR-27b-3p/VEGF-C axis modulates stemness characteristics in cervical cancer cells. *Chin Med J (Engl)*. 2021;134(17):2091-101.
385. Sun Y, Xu T, Cao YW, Ding XQ. Antitumor effect of miR-27b-3p on lung cancer cells via targeting Fzd7. *Eur Rev Med Pharmacol Sci*. 2017;21(18):4113-23.
386. Pan YQ, Huang KS, Chong TH, Li JY. LINC01089 blocks malignant progression of thyroid cancer by binding miR-27b-3p to enhance the FBLN5 protein level. *Discov Oncol*. 2022;13(1):114.
387. Miao W, Li N, Gu B, Yi G, Su Z, Cheng H. MiR-27b-3p suppresses glioma development via targeting YAP1. *Biochem Cell Biol*. 2020;98(4):466-73.

388. Liu L, Hu J, Yu T, You S, Zhang Y, Hu L. miR-27b-3p/MARCH7 regulates invasion and metastasis of endometrial cancer cells through Snail-mediated pathway. *Acta Biochim Biophys Sin (Shanghai)*. 2019;51(5):492-500.
389. Lin XH, Liu ZY, Zhang DY, Zhang S, Tang WQ, Li DP, et al. circRanGAP1/miR-27b-3p/NRAS Axis may promote the progression of hepatocellular Carcinoma. *Exp Hematol Oncol*. 2022;11(1):92.
390. Jusoh AR, Al-Astani Bin Tengku Din TAD, Abdullah-Zawawi MR, Abdul Rahman WFW, Nafi SNM, Romli RC, et al. Unraveling Roles of miR-27b-3p as a Potential Biomarker for Breast Cancer in Malay Women via Bioinformatics Analysis. *Int J Mol Cell Med*. 2023;12(3):257-74.
391. Jiang Q, Xing W, Cheng J, Yu Y. Long Non-Coding RNA TP73-AS1 Promotes the Development of Lung Cancer by Targeting the miR-27b-3p/LAPTM4B Axis. *Onco Targets Ther*. 2020;13:7019-31.
392. Han M, Li N, Li F, Wang H, Ma L. MiR-27b-3p exerts tumor suppressor effects in esophageal squamous cell carcinoma by targeting Nrf2. *Hum Cell*. 2020;33(3):641-51.
393. Dou R, Liu K, Yang C, Zheng J, Shi D, Lin X, et al. EMT-cancer cells-derived exosomal miR-27b-3p promotes circulating tumour cells-mediated metastasis by modulating vascular permeability in colorectal cancer. *Clin Transl Med*. 2021;11(12):e595.
394. Chen B, Liu C, Long H, Bai G, Zhu Y, Xu H. N(6)-methyladenosine-induced long non-coding RNA PVT1 regulates the miR-27b-3p/BLM axis to promote prostate cancer progression. *Int J Oncol*. 2023;62(1).
395. Zhu J, Zou Z, Nie P, Kou X, Wu B, Wang S, et al. Downregulation of microRNA-27b-3p enhances tamoxifen resistance in breast cancer by increasing NR5A2 and CREB1 expression. *Cell Death Dis*. 2016;7(11):e2454.
396. Xu Y, Han YF, Ye B, Zhang YL, Dong JD, Zhu SJ, Chen J. miR-27b-3p is Involved in Doxorubicin Resistance of Human Anaplastic Thyroid Cancer Cells via Targeting Peroxisome Proliferator-Activated Receptor Gamma. *Basic Clin Pharmacol Toxicol*. 2018;123(6):670-7.
397. Sun W, Li J, Zhou L, Han J, Liu R, Zhang H, et al. The c-Myc/miR-27b-3p/ATG10 regulatory axis regulates chemoresistance in colorectal cancer. *Theranostics*. 2020;10(5):1981-96.
398. Roška J, Lobo J, Ivovič D, Wachsmannová L, Mueller T, Henrique R, et al. Integrated Microarray-Based Data Analysis of miRNA Expression Profiles: Identification of Novel Biomarkers of Cisplatin-Resistance in Testicular Germ Cell Tumours. *Int J Mol Sci*. 2023;24(3).
399. Ma SQ, Wang YC, Li Y, Li XY, Yang J, Sheng YM. LncRNA XIST promotes proliferation and cisplatin resistance of oral squamous cell carcinoma by downregulating miR-27b-3p. *J Biol Regul Homeost Agents*. 2020;34(6):1993-2001.
400. Hummel R, Sie C, Watson DI, Wang T, Ansar A, Michael MZ, et al. MicroRNA signatures in chemotherapy resistant esophageal cancer cell lines. *World J Gastroenterol*. 2014;20(40):14904-12.
401. Chen D, Si W, Shen J, Du C, Lou W, Bao C, et al. miR-27b-3p inhibits proliferation and potentially reverses multi-chemoresistance by targeting CBLB/GRB2 in breast cancer cells. *Cell Death Dis*. 2018;9(2):188.
402. Tommasi C, Pellegrino B, Boggiani D, Sikokis A, Michiara M, Uliana V, et al. Biological Role and Clinical Implications of microRNAs in BRCA Mutation Carriers. *Front Oncol*. 2021;11:700853.
403. Yu X, Wu LC, Bowcock AM, Aronheim A, Baer R. The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. *J Biol Chem*. 1998;273(39):25388-92.
404. Gasparri ML, Casorelli A, Bardhi E, Besharat AR, Savone D, Ruscito I, et al. Beyond circulating microRNA biomarkers: Urinary microRNAs in ovarian and breast cancer. *Tumour Biol*. 2017;39(5):1010428317695525.
405. Tanic M, Yanowski K, Gómez-López G, Rodriguez-Pinilla MS, Marquez-Rodas I, Osorio A, et al. MicroRNA expression signatures for the prediction of BRCA1/2 mutation-associated hereditary breast cancer in paraffin-embedded formalin-fixed breast tumors. *Int J Cancer*. 2015;136(3):593-602.
406. Barwal TS, Singh N, Sharma U, Bazala S, Rani M, Behera A, et al. miR-590-5p: A double-edged sword in the oncogenesis process. *Cancer Treat Res Commun*. 2022;32:100593.

407. Salem M, O'Brien JA, Bernaudo S, Shower H, Ye G, Brkić J, et al. miR-590-3p Promotes Ovarian Cancer Growth and Metastasis via a Novel FOXA2-Versican Pathway. *Cancer Res.* 2018;78(15):4175-90.
408. Salem M, Shan Y, Bernaudo S, Peng C. miR-590-3p Targets Cyclin G2 and FOXO3 to Promote Ovarian Cancer Cell Proliferation, Invasion, and Spheroid Formation. *Int J Mol Sci.* 2019;20(8).
409. Plantamura I, Cosentino G, Cataldo A. MicroRNAs and DNA-Damaging Drugs in Breast Cancer: Strength in Numbers. *Front Oncol.* 2018;8:352.
410. Shen J, Ambrosone CB, DiCioccio RA, Odunsi K, Lele SB, Zhao H. A functional polymorphism in the miR-146a gene and age of familial breast/ovarian cancer diagnosis. *Carcinogenesis.* 2008;29(10):1963-6.
411. Sun C, Li N, Yang Z, Zhou B, He Y, Weng D, et al. miR-9 regulation of BRCA1 and ovarian cancer sensitivity to cisplatin and PARP inhibition. *J Natl Cancer Inst.* 2013;105(22):1750-8.
412. Moskwa P, Buffa FM, Pan Y, Panchakshari R, Gottipati P, Muschel RJ, et al. miR-182-mediated downregulation of BRCA1 impacts DNA repair and sensitivity to PARP inhibitors. *Mol Cell.* 2011;41(2):210-20.
413. Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, et al. Requirement of bic/microRNA-155 for normal immune function. *Science.* 2007;316(5824):608-11.
414. Thai TH, Calado DP, Casola S, Ansel KM, Xiao C, Xue Y, et al. Regulation of the germinal center response by microRNA-155. *Science.* 2007;316(5824):604-8.
415. Pessôa-Pereira D, Evangelista AF, Causin RL, da Costa Vieira RA, Abrahão-Machado LF, Santana IVV, et al. miRNA expression profiling of hereditary breast tumors from BRCA1- and BRCA2-germline mutation carriers in Brazil. *BMC Cancer.* 2020;20(1):143.
416. Erturk E, Cecener G, Egeli U, Tunca B, Tezcan G, Gokgoz S, et al. Expression status of let-7a and miR-335 among breast tumors in patients with and without germ-line BRCA mutations. *Mol Cell Biochem.* 2014;395(1-2):77-88.
417. Elias K, Smyczynska U, Stawiski K, Nowicka Z, Webber J, Kaplan J, et al. Identification of BRCA1/2 mutation female carriers using circulating microRNA profiles. *Nat Commun.* 2023;14(1):3350.
418. Saçar Demirci MD. Computational Detection of Pre-microRNAs. *Methods Mol Biol.* 2022;2257:167-74.
419. Zhang Y, Verbeek FJ. Comparison and integration of target prediction algorithms for microRNA studies. *J Integr Bioinform.* 2010;7(3).
420. Peterson SM, Thompson JA, Ufkin ML, Sathyanarayana P, Liaw L, Congdon CB. Common features of microRNA target prediction tools. *Frontiers in Genetics.* 2014;5.
421. Bereczki Z, Benczik B, Balogh OM, Marton S, Puhl E, Pétervári M, et al. Mitigating off-target effects of small RNAs: conventional approaches, network theory and artificial intelligence. *Br J Pharmacol.* 2024.
422. Zhao B, Xue B. Significant improvement of miRNA target prediction accuracy in large datasets using meta-strategy based on comprehensive voting and artificial neural networks. *BMC Genomics.* 2019;20(1):158.
423. Maqsood MI, Matin MM, Bahrami AR, Ghasroldasht MM. Immortality of cell lines: challenges and advantages of establishment. *Cell Biol Int.* 2013;37(10):1038-45.
424. Idrisova KF, Simon HU, Gomzikova MO. Role of Patient-Derived Models of Cancer in Translational Oncology. *Cancers (Basel).* 2022;15(1).
425. Skok K, Gradišnik L, Maver U, Kozar N, Sobočan M, Takač I, et al. Gynaecological cancers and their cell lines. *J Cell Mol Med.* 2021;25(8):3680-98.
426. Tiberio P, Callari M, Angeloni V, Daidone MG, Appierto V. Challenges in Using Circulating miRNAs as Cancer Biomarkers. *BioMed Research International.* 2015;2015(1):731479.
427. DiNardo CD, Korde LA, Yurgelun MB. A Case-Based Approach to Understanding Complex Genetic Information in an Evolving Landscape. *Am Soc Clin Oncol Educ Book.* 2021;41:1-11.

428. Consortium BCL. Cancer risks in BRCA2 mutation carriers. *J Natl Cancer Inst.* 1999;91(15):1310-6.
429. Foruzandeh Z, Zeinali-Sehrig F, Nejati K, Rahmanpour D, Pashazadeh F, Seif F, Alivand MR. CircRNAs as potent biomarkers in ovarian cancer: a systematic scoping review. *Cell Mol Biol Lett.* 2021;26(1):41.
430. Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L. Complementary sequence-mediated exon circularization. *Cell.* 2014;159(1):134-47.
431. Huang Y, Zhu Q. Mechanisms Regulating Abnormal Circular RNA Biogenesis in Cancer. *Cancers.* 2021;13(16):4185.
432. Naumann M, Pal A, Goswami A, Lojewski X, Japtok J, Vehlouw A, et al. Impaired DNA damage response signaling by FUS-NLS mutations leads to neurodegeneration and FUS aggregate formation. *Nature Communications.* 2018;9(1):335.
433. Han K, Wang FW, Cao CH, Ling H, Chen JW, Chen RX, et al. CircLONP2 enhances colorectal carcinoma invasion and metastasis through modulating the maturation and exosomal dissemination of microRNA-17. *Mol Cancer.* 2020;19(1):60.
434. Liu S, Yang N, Jiang X, Wang J, Dong J, Gao Y. FUS-induced circular RNA ZNF609 promotes tumorigenesis and progression via sponging miR-142-3p in lung cancer. *Journal of cellular physiology.* 2021;236(1):79-92.
435. Chen T, Wang X, Li C, Zhang H, Liu Y, Han D, et al. CircHIF1A regulated by FUS accelerates triple-negative breast cancer progression by modulating NFIB expression and translocation. *Oncogene.* 2021;40(15):2756-71.
436. Mirabelli P, Coppola L, Salvatore M. Cancer Cell Lines Are Useful Model Systems for Medical Research. *Cancers (Basel).* 2019;11(8).
437. Masters JR. Human cancer cell lines: fact and fantasy. *Nat Rev Mol Cell Biol.* 2000;1(3):233-6.
438. Lorsch JR, Collins FS, Lippincott-Schwartz J. Cell Biology. Fixing problems with cell lines. *Science.* 2014;346(6216):1452-3.
439. Zaaijer S, Groen SC, Sanjana NE. Tracking cell lineages to improve research reproducibility. *Nat Biotechnol.* 2021;39(6):666-70.
440. Gold M. A Conspiracy of Cells: One Woman's Immortal Legacy-And the Medical Scandal It Caused: State University of New York Press, 1985; 1986 1st January 1986.
441. Capes-Davis A, Reid YA, Kline MC, Storts DR, Strauss E, Dirks WG, et al. Match criteria for human cell line authentication: where do we draw the line? *Int J Cancer.* 2013;132(11):2510-9.
442. Coward J, Kulbe H, Chakravarty P, Leader D, Vassileva V, Leinster DA, et al. Interleukin-6 as a therapeutic target in human ovarian cancer. *Clin Cancer Res.* 2011;17(18):6083-96.
443. Mohl DA, Lagies S, Lonzer A, Pfäffle SP, Groß P, Benka M, et al. On the Quest for Biomarkers: A Comprehensive Analysis of Modified Nucleosides in Ovarian Cancer Cell Lines. *Cells.* 2025;14(9):626.
444. Elias KM, Emori MM, Papp E, MacDuffie E, Konecny GE, Velculescu VE, Drapkin R. Beyond genomics: Critical evaluation of cell line utility for ovarian cancer research. *Gynecologic Oncology.* 2015;139(1):97-103.
445. Barnes BM, Nelson L, Tighe A, Burghel GJ, Lin IH, Desai S, et al. Distinct transcriptional programs stratify ovarian cancer cell lines into the five major histological subtypes. *Genome Med.* 2021;13(1):140.
446. Byrum AK, Vindigni A, Mosammaparast N. Defining and Modulating 'BRCAness'. *Trends Cell Biol.* 2019;29(9):740-51.
447. Passaro A, Al Bakir M, Hamilton EG, Diehn M, André F, Roy-Chowdhuri S, et al. Cancer biomarkers: Emerging trends and clinical implications for personalized treatment. *Cell.* 2024;187(7):1617-35.
448. Valeri N, Braconi C, Gasparini P, Murgia C, Lampis A, Paulus-Hock V, et al. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. *Cancer Cell.* 2014;25(4):469-83.

449. Xia X, Yang B, Zhai X, Liu X, Shen K, Wu Z, Cai J. Prognostic role of microRNA-21 in colorectal cancer: a meta-analysis. *PLoS One*. 2013;8(11):e80426.
450. FDA grants breakthrough designation to urine-based liquid biopsy for prostate cancer [press release]. 26/10/2025 2020.
451. Lin J, Lyu Z, Feng H, Xie H, Peng J, Zhang W, et al. CircPDIA3/miR-449a/XBP1 feedback loop curbs pyroptosis by inhibiting palmitoylation of the GSDME-C domain to induce chemoresistance of colorectal cancer. *Drug Resistance Updates*. 2024;76:101097.
452. Niculescu AB, Le-Niculescu H. Precision medicine in psychiatry: biomarkers to the forefront. *Neuropsychopharmacology*. 2022;47(1):422-3.
453. Dodbele S, Mutlu N, Wilusz JE. Best practices to ensure robust investigation of circular RNAs: pitfalls and tips. *EMBO Rep*. 2021;22(3):e52072.
454. Li X, Jiang X, Lu J, Lin Y, Jiang L, Li Y, et al. CircCERS6 Suppresses the Development of Epithelial Ovarian Cancer Through Mediating miR-630/RASSF8. *Biochem Genet*. 2022;60(6):2611-29.
455. Zhang L, Chen H, He F, Zhang S, Li A, Zhang A, Zhang A. MicroRNA-320a Promotes Epithelial Ovarian Cancer Cell Proliferation and Invasion by Targeting RASSF8. *Front Oncol*. 2021;11:581932.
456. Huang X, Tan W, Liu Z, Fu X, Li Z, Lai S, et al. EIF4A3-induced circZFAND6 promotes breast cancer proliferation and metastasis through the miR-647/FASN axis. *Life Sciences*. 2023;324:121745.
457. Zheng X, Huang M, Xing L, Yang R, Wang X, Jiang R, et al. The circRNA circSEPT9 mediated by E2F1 and EIF4A3 facilitates the carcinogenesis and development of triple-negative breast cancer. *Molecular cancer*. 2020;19(1):73.
458. Wang R, Zhang S, Chen X, Li N, Li J, Jia R, et al. EIF4A3-induced circular RNA MMP9 (circMMP9) acts as a sponge of miR-124 and promotes glioblastoma multiforme cell tumorigenesis. *Molecular cancer*. 2018;17(1):166.
459. Jiang Z, Tai Q, Xie X, Hou Z, Liu W, Yu Z, et al. EIF4A3-induced circ_0084615 contributes to the progression of colorectal cancer via miR-599/ONECUT2 pathway. *Journal of Experimental & Clinical Cancer Research*. 2021;40(1):227.
460. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. *Nat Rev Cancer*. 2013;13(10):714-26.
461. Barr MP, Gray SG, Hoffmann AC, Hilger RA, Thomale J, O'Flaherty JD, et al. Generation and characterisation of cisplatin-resistant non-small cell lung cancer cell lines displaying a stem-like signature. *PLoS One*. 2013;8(1):e54193.
462. Xavier CP, Pesic M, Vasconcelos MH. Understanding Cancer Drug Resistance by Developing and Studying Resistant Cell Line Models. *Curr Cancer Drug Targets*. 2016;16(3):226-37.
463. Polajžer S, Černe K. Precision Medicine in High-Grade Serous Ovarian Cancer: Targeted Therapies and the Challenge of Chemoresistance. *Int J Mol Sci*. 2025;26(6).
464. Wu M, Swartz MA. Modeling tumor microenvironments in vitro. *J Biomech Eng*. 2014;136(2):021011.
465. Fong EL, Harrington DA, Farach-Carson MC, Yu H. Heralding a new paradigm in 3D tumor modeling. *Biomaterials*. 2016;108:197-213.
466. Hu M, Zhou C, Li M, Zhao J. From 3D culture to clinical decision-making: Systematic innovations in breast cancer organoids. *Biomater Adv*. 2026;179:214528.
467. HSE. CARBOplatin (AUC5-7.5) and PACLitaxel 175mg/m² Therapy 2016 [Available from: <https://www.hse.ie/eng/services/list/5/cancer/profinfo/chemoprotocols/gynaecology/303-carboplatin-auc5-7-5-and-paclitaxel-175mg-m2-therapy.pdf>].
468. Luo J, Ou S, Wei H, Qin X, Jiang Q. Comparative Efficacy and Safety of Poly (ADP-Ribose) Polymerase Inhibitors in Patients With Ovarian Cancer: A Systematic Review and Network Meta-Analysis. *Front Oncol*. 2022;12:815265.
469. GraphPadSoftware. Molarity Calculator. GraphPad QuickCalcs. 2025 [Available from: <https://www.graphpad.com/quickcalcs/molarityform/>].

470. Pignata S, Scambia G, Katsaros D, Gallo C, Pujade-Lauraine E, De Placido S, et al. Carboplatin plus paclitaxel once a week versus every 3 weeks in patients with advanced ovarian cancer (MITO-7): a randomised, multicentre, open-label, phase 3 trial. *Lancet Oncol.* 2014;15(4):396-405.
471. Mahmood RD, Morgan RD, Edmondson RJ, Clamp AR, Jayson GC. First-Line Management of Advanced High-Grade Serous Ovarian Cancer. *Curr Oncol Rep.* 2020;22(6):64.
472. Chan JK, Brady MF, Penson RT, Huang H, Birrer MJ, Walker JL, et al. Weekly vs. Every-3-Week Paclitaxel and Carboplatin for Ovarian Cancer. *N Engl J Med.* 2016;374(8):738-48.
473. Kim SI, Lee M, Kim HS, Chung HH, Kim JW, Park NH, Song YS. Effect of BRCA mutational status on survival outcome in advanced-stage high-grade serous ovarian cancer. *J Ovarian Res.* 2019;12(1):40.
474. Shahzad M, Naci H, Esselen KM, Dottino JA, Wagner AK. Regulatory histories of recently withdrawn ovarian cancer treatment indications of 3 PARP inhibitors in the US and Europe: lessons for the accelerated approval pathway. *J Pharm Policy Pract.* 2024;17(1):2351003.
475. Fedier A, Maggi N, Tozzi A, Disler M, Coelho R, Jacob F, Heinzelmann-Schwarz V. Exposure to escalating olaparib does not induce acquired resistance to PARPi and to other chemotherapeutic compounds in ovarian cancer cell lines. *Int J Oncol.* 2022;61(1).
476. Moore K, Colombo N, Scambia G, Kim B-G, Oaknin A, Friedlander M, et al. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *New England Journal of Medicine.* 2018;379(26):2495-505.
477. Colombo N, Moore K, Scambia G, Oaknin A, Friedlander M, Lisyanskaya A, et al. Tolerability of maintenance olaparib in newly diagnosed patients with advanced ovarian cancer and a BRCA mutation in the randomized phase III SOLO1 trial. *Gynecol Oncol.* 2021;163(1):41-9.
478. Rose PG, Yao M, Chambers LM, Mahdi H, DeBernardo R, Michener CM, et al. PARP inhibitors decrease response to subsequent platinum-based chemotherapy in patients with BRCA mutated ovarian cancer. *Anticancer Drugs.* 2021;32(10):1086-92.
479. Colombo N, Tomao F, Benedetti Panici P, Nicoletto MO, Tognon G, Bologna A, et al. Randomized phase II trial of weekly paclitaxel vs. cediranib-olaparib (continuous or intermittent schedule) in platinum-resistant high-grade epithelial ovarian cancer. *Gynecol Oncol.* 2022;164(3):505-13.
480. Frenel JS, Kim JW, Aryal N, Asher R, Berton D, Vidal L, et al. Efficacy of subsequent chemotherapy for patients with BRCA1/2-mutated recurrent epithelial ovarian cancer progressing on olaparib versus placebo maintenance: post-hoc analyses of the SOLO2/ENGOT Ov-21 trial. *Ann Oncol.* 2022;33(10):1021-8.
481. Mittica G, Ghisoni E, Giannone G, Genta S, Aglietta M, Sapino A, Valabrega G. PARP Inhibitors in Ovarian Cancer. *Recent Pat Anticancer Drug Discov.* 2018;13(4):392-410.
482. Busschots S, O'Toole S, O'Leary JJ, Stordal B. Carboplatin and taxol resistance develops more rapidly in functional BRCA1 compared to dysfunctional BRCA1 ovarian cancer cells. *Exp Cell Res.* 2015;336(1):1-14.
483. Makowska A, Kontny U, Weiskirchen R. HeLa cells cross-contaminated nasopharyngeal carcinoma cell lines: Still a common problem. *Br J Cancer.* 2024;130(12):1885-6.
484. Yang M, He J, Xia S, Wang Y, Xiong J, Liao C, et al. Investigation of the mixed origins of the MGC-803 cell line reveals that it is a hybrid cell line derived from HeLa. *Hum Cell.* 2024;37(2):560-6.
485. Zhang J, Wang C, Jia C, Zhang Y, Qing X, Zhang Y, et al. The Role of Circular RNAs in the Physiology and Pathology of the Mammalian Ovary. *Int J Mol Sci.* 2022;23(23).
486. Qin K, Fenghua Z, Wang H, Wang N, Qiu H, Jia X, et al. circRNA circSnx12 confers Cisplatin chemoresistance to ovarian cancer by inhibiting ferroptosis through a miR-194-5p/SLC7A11 axis. *BMB Rep.* 2023.
487. Zhao Z, Ji M, Wang Q, He N, Li Y. Circular RNA Cdr1as Upregulates SCA1 to Suppress Cisplatin Resistance in Ovarian Cancer via miR-1270 Suppression. *Mol Ther Nucleic Acids.* 2019;18:24-33.

488. Xia B, Zhao Z, Wu Y, Wang Y, Zhao Y, Wang J. Circular RNA circTNPO3 Regulates Paclitaxel Resistance of Ovarian Cancer Cells by miR-1299/NEK2 Signaling Pathway. *Mol Ther Nucleic Acids*. 2020;21:780-91.
489. Sheng R, Li X, Wang Z, Wang X. Circular RNAs and their emerging roles as diagnostic and prognostic biomarkers in ovarian cancer. *Cancer Lett*. 2020;473:139-47.
490. Ghafouri-Fard S, Khoshbakht T, Hussen BM, Taheri M, Samsami M. Emerging role of circular RNAs in the pathogenesis of ovarian cancer. *Cancer Cell Int*. 2022;22(1):172.
491. Tool BVD. Area-Proportional Venn Diagram Plotter and Editor 2025 [Available from: <https://bioinforx.com/apps/venn.php>].
492. Yalan S, Yanfang L, He C, Yujie T. Circular RNA circRHOBTB3 inhibits ovarian cancer progression through PI3K/AKT signaling pathway. *Panminerva Med*. 2020.
493. Cheng H, Wang N, Tian J, Li Y, Ren L, Shi Z. Circular RNA Circ_0025033 Promotes the Evolvement of Ovarian Cancer Through the Regulation of miR-330-5p/KLK4 Axis. *Cancer Manag Res*. 2020;12:2753-65.
494. Wang H, Zhang X, Qiao L, Wang H. CircRNA circ_0000554 promotes ovarian cancer invasion and proliferation by regulating miR-567. *Environ Sci Pollut Res Int*. 2022;29(13):19072-80.
495. Zhang F, Xu Y, Ye W, Jiang J, Wu C. Circular RNA S-7 promotes ovarian cancer EMT via sponging miR-641 to up-regulate ZEB1 and MDM2. *Biosci Rep*. 2020;40(7).
496. Zhou J, Dong ZN, Qiu BQ, Hu M, Liang XQ, Dai X, et al. CircRNA FGFR3 induces epithelial-mesenchymal transition of ovarian cancer by regulating miR-29a-3p/E2F1 axis. *Aging (Albany NY)*. 2020;12(14):14080-91.
497. Zhang N, Wang X, Li Y, Lu Y, Sheng C, Sun Y, et al. Mechanisms and therapeutic implications of gene expression regulation by circRNA-protein interactions in cancer. *Communications Biology*. 2025;8(1):77.
498. Chen S, Zhang J, Zhao F. Screening Linear and Circular RNA Transcripts from Stress Granules. *Genomics Proteomics Bioinformatics*. 2023;21(4):886-93.
499. Liu W, Niu J, Huo Y, Zhang L, Han L, Zhang N, Yang M. Role of circular RNAs in cancer therapy resistance. *Molecular Cancer*. 2025;24(1):55.
500. Lagunas-Rangel FA. Role of circular RNAs in DNA repair. *RNA Biology*. 2024;21(1):1226-38.
501. Wang Z, Ge Q, Anwaier A, Liu S, Tian X, Zhang Z, et al. Hsa_circ_0038737 promotes PARPi resistance in castration-resistant prostate cancer via IGF2BP3-mediated DNPH1 mRNA stabilization. *Mol Cancer*. 2025;24(1):238.
502. Yan Y, Wang H, Hu J, Guo T, Dong Q, Yin H, et al. CircRNA-104718 promotes glioma malignancy through regulation of miR-218-5p/HMGB1 signalling pathway. *Metab Brain Dis*. 2023;38(5):1531-42.
503. Yu J, Yang M, Zhou B, Luo J, Zhang Z, Zhang W, Yan Z. CircRNA-104718 acts as competing endogenous RNA and promotes hepatocellular carcinoma progression through microRNA-218-5p/TXNDC5 signaling pathway. *Clin Sci (Lond)*. 2019;133(13):1487-503.
504. Ding J, Wang Q, Guo N, Wang H, Chen H, Ni G, Li P. CircRNA circ_0072995 promotes the progression of epithelial ovarian cancer by modulating miR-147a/CDK6 axis. *Aging (Albany NY)*. 2020;12(17):17209-23.
505. Guo M, Li S, Zhao X, Yuan Y, Zhang B, Guan Y. Knockdown of Circular RNA Hsa_circ_0000714 Can Regulate RAB17 by Sponging miR-370-3p to Reduce Paclitaxel Resistance of Ovarian Cancer Through CDK6/RB Pathway. *Onco Targets Ther*. 2020;13:13211-24.
506. Pei C, Wang H, Shi C, Zhang C, Wang M. CircRNA hsa_circ_0013958 may contribute to the development of ovarian cancer by affecting epithelial-mesenchymal transition and apoptotic signaling pathways. *J Clin Lab Anal*. 2020;34(7):e23292.
507. Wang Z, Zhang W, Fang J, Xie P, Miao M, Yang H. Circular RNA circEXOC6B Inhibits the Progression of Ovarian Cancer by Sponging miR-421 and Regulating RUS1 Expression. *Onco Targets Ther*. 2020;13:8233-43.

508. Wei X, Lv H, Yang S, Yang X. CircRNA PLOD2 enhances ovarian cancer propagation by controlling miR-378. *Saudi J Biol Sci.* 2021;28(11):6260-5.
509. Zhao Y, Hu Y, Shen Q, Chen Q, Zhu XJ, Jiang SS, Zhang Q. CircRNA_MYLK promotes malignant progression of ovarian cancer through regulating microRNA-652. *Eur Rev Med Pharmacol Sci.* 2020;24(10):5281-91.
510. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet.* 2019;20(11):675-91.

11 Appendix

Appendix 1 : Custom designed Primer sequence

Gene	Associated miRNA	Customer Designed Primers
Mycoplasma	n/a	FP1: 5'-GGGAGCAAACAGGATTAGATACCT -3' RP1: 5'-TGCACCATCTGTCACTCTGTTAACCTC -3'
HMGA2	hsa-miR-205-5p	FP1 5'- AACAAGTCCTTCATTCCACCGGA -3' RP1 5'- AACAATTCCTTCATTCCACCGGA -3'
ZNF253	hsa-miR-590-3p	FP1 5'- CGCCGGTACTTTTATGGATAAGC -3' FP2 5'- CGCGGCCTACTTTTATGGATAAG -3'
GCC2	hsa-miR-27b-3p	FP1 5'- CGCCGGTACTTTTATGGATAAGC -3' FP2 5'- CGCGGCCTACTTTTATGGATAAG -3'
HMGA2	hsa-let-7f-5p	FP1 5'- AACAAGTCCTTCATTCCACCGGA -3' FP2 5'- AACAAGTCCTTCATTCCACCGGA -3'
HMGA2	hsa-miR-98-5p	FP1 5'- AATCGGCGTGAGGTAGTAAGTTG -3' FP2 5'- AGGCGCTGAGGTAGTAAGTTGTA-3'
C17orf62	hsa-miR-3176	FP1 5'- AACAAGACTGGCCTGGGACTAC -3' FP2 5'- AACGATAACTGGCCTGGGACTAC-3'
RRAGD	hsa-miR-3613	FP1 5'- ATGCGCGCTGTTGTACTTTTTT -3' FP2 5'- CGCGGCCTGTTGTACTTTTTTT-3'
ZNF460	hsa-let-7a-3p	FP1 5'- ATGCGCGCCTATACAATCTACTG -3' FP2 5'- CGCCGGCTATACAATCTACTGTC -3'
ZNF791	hsa-miR-652-3p	FP1 5'- AACCTCCAATGGCGCCACTA -3' FP2 5'- AAGCACAATGGCGCCACTAG -3'

Appendix 2 : Case Report Form (CRF) for CAMPAIGN Registry

Participant Study ID: _____

Date of Form Completion: _____

Completed by (Name): _____

Completed by (Role): _____

Date: _____

Please Note: This form is to be completed only for participants who have provided informed consent to participate in this study. It is essential that only individuals who have explicitly agreed to contribute their data and information complete this form. Consent must be obtained prior to the collection of any personal, medical, or genetic data. If consent is not given, the form should not be filled out, and no data should be collected or entered. Ensuring that all participants have consented to their involvement in the study is a fundamental requirement for the ethical conduct of this research.

1. Demographic Information

Field	Information
Age	
Gender	
Ethnicity	
Geographic Location of birth	

2. Clinical Records

Field	Information
Type of Cancer: (please select more than one if relevant)	☐ Breast ☐ Ovarian ☐ Colorectal ☐ Prostate ☐ Other (please specify):
Stage at Diagnosis: (If multiple diagnoses, please state stage of each cancer)	
Age at Diagnosis: (If multiple diagnoses, please age each cancer was diagnosed)	
Treatment Modalities Received:	☐ Surgery ☐ Chemotherapy ☐ Radiation Therapy ☐ Immunotherapy ☐ Other (please specify):
Treatment Outcomes:	☐ Complete Remission ☐ Partial Remission ☐ Stable Disease ☐ Progression ☐ Other (please specify):

3. Genetic Profiles

Field	Information
Genetic Alterations Identified:	☐ BRCA1 ☐ BRCA2 ☐ TP53 ☐ Lynch Syndrome (including associated genes such as MLH1, MSH2, MSH6, PMS2) ☐ Other (please specify): _____
Details of Genetic Variants:	_____ _____ _____

4. Genetic Testing Information

Field	Information
Types of Genetic Testing Performed:	☐ BRCA Gene Testing (Germline) ☐ BRCA Gene Testing (Somatic) ☐ High-Risk Breast Panel ☐ HRD (Homologous Recombination Deficiency) ☐ FoundationOne ☐ Other (please specify): _____
Who Performed the Genetic Test:	☐ Public System Referral ☐ Private System Referral ☐ Paid Independently for Testing
Testing Facility or Laboratory:	_____ _____
Date of Testing:	_____ _____

5. Family Medical History

Field	Information
Relatives Diagnosed with Cancer:	Relative 1: Type of Cancer: _____ Relationship to Participant: _____ Age at Diagnosis: _____ Age at Initial Diagnosis: _____ Relative 2: Type of Cancer: _____

Field	Information
	Relationship to Participant: _____ Age at Diagnosis: _____ Age at Initial Diagnosis: _____ Additional Relatives (if applicable): _____ _____ _____
Pathogenic Genetic Variants in Family:	☐ BRCA1 ☐ BRCA2 ☐ TP53 ☐ Lynch Syndrome (including associated genes such as MLH1, MSH2, MSH6, PMS2) ☐ Other (please specify): _____
Family Members Who Have Undergone Genetic Testing:	Family Member 1: Test Results: _____ Family Member 2: Test Results: _____ Additional Family Members (if applicable): _____ _____ _____
Age of Siblings (if applicable):	_____ _____ _____ _____
Size of Family Unit:	_____ _____

6. Screening Information

6.1 Screening Related to Current Diagnosis

Field	Information
Was the cancer diagnosis identified through screening or symptoms?	☐ Screening ☐ Symptoms
If identified through screening, specify the type of screening tests:	☐ Mammogram ☐ FOB Test (Fecal Occult Blood Test) ☐ Colonoscopy ☐ Radiological Screening (e.g., CT scans, PET scans, MRI) ☐ Other (please specify): _____
Type of Screening:	☐ Universal Screening ☐ High-Risk Family Screening

6.2 Current Screening Practices

Field	Information
Is the patient currently undergoing screening?	☐ Yes ☐ No
If yes, specify the type of screening tests currently being performed:	☐ Mammogram ☐ FOB Test (Fecal Occult Blood Test) ☐ Colonoscopy ☐ Radiological Screening (e.g., CT scans, PET scans, MRI) ☐ Other (please specify): _____
Frequency of Current Screening:	☐ Annually ☐ Biannually ☐ Every 3 years ☐ Other (please specify): _____

7. Prophylactic Surgeries

Field	Information
Prophylactic Surgeries Performed:	☐ Risk-Reducing Mastectomy ☐ Risk-Reducing Hysterectomy ☐ Risk-Reducing Colectomy ☐ Other (please specify): _____
Age at Prophylactic Surgery:	_____
Time from Diagnosis to Surgery (in months):	_____
Details of Prophylactic Surgery:	_____ _____ _____

8. Treatment History

Field	Information
Types of Treatments Received:	☐ Surgery ☐ Chemotherapy ☐ Radiation Therapy ☐ Immunotherapy ☐ Other (please specify): _____
Outcomes of Treatments:	_____ _____ _____

9. Additional Relevant Factors

Field	Information
Comorbidities:	
Lifestyle Factors:	Smoking History: ☐ Current Smoker ☐ Former Smoker ☐ Never Smoked Details:
Environmental Exposures:	
Alcohol Intake (Units per Week):	

10. Social History

Field	Information
Number of Children:	

Notes/Comments:[illegible]

Appendix 3 : Patient Information Leaflet for CAMPAIGN Registry

Patient Information Leaflet (Adults)

Protocol Title:

Cancer Predisposing Genetic Alterations Registry : A Pilot Study (CAmPAIGn Study)

Principal Investigator's Name: Prof Karen Cadoo

Telephone No. of Principal Investigator: 01-4103000

Data Controller: St James's Hospital

Contact details for data protection officers: dpo@stjames.ie or dpo@tcd.ie

You are being invited to take part in an optional research study carried out at St. James's Hospital. Before you decide whether or not you wish to take part, you should read the information provided below carefully and if you wish discuss it with your family, friends or GP. Take time to ask questions. Do not feel rushed or under any obligation to decide quickly. You should clearly understand clearly the risks and benefits of participating in this study so that you can make a decision that is right for you. This process is known as Informed Consent.

You are not obliged to take part in this study and if you do not take part, it will have no effect on your care now or in the future. If you decide to take part, you can change your mind at any time without having to give a reason and without any effect on the care you will receive from the medical staff.

WHY IS THIS STUDY BEING DONE?

WHAT IS THE 'CANCER PREDISPOSING GENETIC ALTERATIONS REGISTRY' AND WHY IS IT BEING DONE?

This pilot study serves as a crucial step towards establishing a registry for individuals with cancer predisposing genetic alterations in Ireland. At present, there is a notable absence of a national registry for such patients, highlighting a significant gap in healthcare infrastructure. The primary objective of this initiative is to assess the feasibility and efficacy of implementing such a registry. Registries play a pivotal role in conducting long-term studies aimed at gaining deeper insights into diseases, identifying relevant markers, and evaluating the effectiveness of healthcare interventions. Your participation in this pilot study contributes valuable data that can potentially enhance cancer treatment strategies and facilitate more informed healthcare planning on a broader scale.

Successful registries in Ireland such as the Registries for Cystic Fibrosis have become a model for accelerating progress. A registry differs from a database to keep records of patients contact details with a particular disease, a study record used to answer a single scientific question for a single research question, or a forums. Instead, a well-designed registry has much broader functions, in that it can potentially serve as the foundation for multiple studies from different disciplines that serve many stakeholders. This registry is for patients who have been identified as having a cancer predisposing genetic alteration.

This pilot registry may work with many "Collaboration Partners" internationally, including national registries, national health authorities, and researchers from scientific/clinical institutions. Therefore, we also request your consent to share your data with these partners to fulfil the purpose described above. The registry may also work with the European Medicines Agency (EMA; www.ema.europa.eu/ema), national health authorities, Health Technology Assessment bodies, and the marketing authorisation holders (MAHs; the pharmaceutical companies owning the therapies that patients like you may be receiving).

WHO IS INVITED TO TAKE PART?

Patients who have been identified as having a Cancer Predisposing Genetic Alteration are invited to share their data. This can include people with cancer or people who do not have a cancer diagnosis.

WHO IS ORGANISING AND FUNDING THIS STUDY?

This study is being done in St. James's Hospital Dublin. The project is currently funded by the pharmaceutical company Pfizer.

HOW WILL IT BE CARRIED OUT?

This study will begin in St. James's Hospital in 2024. People who have a cancer predisposing genetic alteration are being invited to take part.

WHAT WILL HAPPEN TO ME IF I AGREE TO TAKE PART?

Your consent authorises the research team to access your medical records, collect and record your medical information. The research team(s) or approved data manager take the relevant medical information from paper-based and electronic hospital(s) records, and information on healthcare services used from the Health Service Executive (HSE). Keeping participants records up-to-date requires a lot of work and takes time. You may also be contacted by the data manager by phone for clarification of the data. Your data can be updated with any pertinent clinical developments.

WHAT INFORMATION WILL BE COLLECTED?

Information on your diagnosis, genetics, cancer diagnosis, screening tests, health complications, medicines and treatments, hospitalisations, healthcare services use and information about how the condition and treatments have an impact on you.

ARE THERE ANY EXTRA COSTS INVOLVED IF YOU DECIDE TO TAKE PART IN THE REGISTRY?

No extra costs are involved related to sharing your data and nor will you receive any payment for sharing their data with the Registry.

BENEFITS:

If you take part in the study and agree for your data to be collected, no direct benefits can be promised. However, the results may help scientists and doctors understand the significance of these alterations. It may help us to understand more clearly how these alterations impact your health. Further, by obtaining data we can estimate both the number of people effected and how they are effected. This gives us information which we can use to advocate or plan for services for the group. This knowledge may improve treatment for cancer patients in the future.

RISKS:

The study involves sharing your data with the research group. The research group employs multiple strategies including implementing the appropriate technical and organisational measures to ensure the highest security standards (see below on Confidentiality and Privacy Protection). However, there is a risk that the registry may suffer a data breach. A data breach occurs when the data for which your company/organisation is responsible suffers a security incident resulting in a breach of confidentiality, availability or integrity. If a data breach was to result in a risk to the rights and freedoms of patients, affected individuals would be informed

CONFIDENTIALITY

We understand that information about you obtained in connection with your health is personal, and we are committed to protecting the privacy of that information. If you decide to participate in this study, the study staff will get the information that identifies you (such as name, medical record number, date of birth). This information will be de-identified with a (pseudo-anonymised) by removing certain identifiable data, and providing each participant with a study number or code prior to collating the data. The key matching your identifiable date with the study code will be kept in a secure location and available only to the Principle Investigator and select members of the study team. Any information that can identify you will remain confidential.

PROTECTING YOUR PRIVACY IS EXTREMELY IMPORTANT TO US.

Your data will be processed in accordance with this consent and Irish data protection law. We are accountable under The EU General Data Protection Regulations 2018, to demonstrate compliance to data protection principles when collecting, processing, storing and sharing your data.

The study has very strict governance procedures in place. It has been approved by Tallaght University Hospital and St. James's Hospital Joint Research Ethics Committee and each of the

following departments at St. James's Hospital; The Office of Research & Innovation, The Department of Legal & Insurance and The Data Protection Office. Trinity's Data Protection Office has also reviewed the study from a data protection perspective.

Your data will be treated with the highest standards of security and confidentiality. Researchers undergo data protection training and sign a confidentiality agreement. The study has many arrangements in place to keep your data safe including restricted access to buildings, offices and computers. Databases are password protected. A Data Protection Impact Assessment (DPIA) has also been carried out. DPIAs help to identify potential risks and to minimise these risks. You can find out more about DPIAs on the Data Protection Commissioner website:

<https://www.dataprotection.ie/en/organisations/know-your-obligations/data-protection-impact-assessments>

WHAT TYPE OF DATA WILL BE COLLECTED?

The study will collect data from your hospital chart, hospital electronic patient records and other hospital databases. Only the study team will know your identity so that they can follow your care and treatment.

The study will collect and store the following information:

Name, address, date of birth, hospital number, lab identifiers

Results: radiology (X-ray, CT, MRI), histology if appropriate (type of cancer), genetic & molecular diagnostic tests

Treatments: any medicines given to you and how you responded

Medical history: previous illnesses, treatments received, medicines, medical procedures

WHAT ARE YOUR RIGHTS?

Under GDPR (General Data Protection Regulation) you have rights. The study has data protection measures in place and is committed to ensuring that your rights under GDPR are protected. To find out more about your rights go to: <http://www.stjames.ie/InformationGovernance/PrivacyPolicyFull/>

St James's Hospital and Trinity College Dublin are the data controllers for this study. The study will use your health data to help with scientific research (in the public interest). We also ask for your

consent as a data protection safeguard, in accordance with the Irish Health Research Regulations 2018.

WILL I BE TOLD ABOUT THE RESEARCH RESULTS?

Unfortunately for various reasons it is not practical to notify each person the general research outputs. However, research results are published in medical & scientific journals and conveyed as scientific presentations at national and international conferences. These results will hopefully add to the body of knowledge to help improve the ways we diagnose and treat our patients. The privacy and confidentiality of all patients will be maintained throughout the research.

IF YOU REQUIRE FURTHER INFORMATION

If you have any further questions about the study, or if you wish to withdraw from the study you may do so without giving a reason and your future treatment will not be affected.

For additional information now or any future time please contact:

Name: Prof Karen Cadoo

Address Genetics Department, St. James's Hospital Phone No: 01-4103000

Appendix 4 : Non-Significant Genes Identified in UALCAN TCGA Dataset Interrogation

GENE RANK to miRNA	Gene	Expression on Survival	Expression of gene according to stages (UALCAN), statistical significance		
		p Value	Stage2-vs-Stage3	Stage2-vs-Stage4	Stage3-vs- Stage4
3	<i>AKIRIN1</i>	Nos	Nos	Nos	Nos
5	<i>ANKRD45</i>	Nos	Nos	Nos	Nos
2	<i>ARID3B</i>	Nos	Nos	Nos	Nos
1	<i>BDNF</i>	Nos	Nos	Nos	Nos
5	<i>C11orf86</i>	Nos	Nos	Nos	Nos
1	<i>C17orf62</i>	Nos	Nos	Nos	Nos
4	<i>CAPZB</i>	Nos	Nos	Nos	Nos
2	<i>CCL7</i>	Nos	Nos	Nos	Nos
3	<i>CHN1</i>	Nos	Nos	Nos	Nos
3	<i>CRLF3</i>	Nos	Nos	Nos	Nos
5	<i>CST3</i>	Nos	Nos	Nos	Nos
4	<i>FIGN</i>	Nos	Nos	Nos	Nos
1	<i>GCC2</i>	Nos	Nos	Nos	Nos
1	<i>HMGA2</i>	Nos	Nos	Nos	Nos
5	<i>HOXA3</i>	Nos	Nos	Nos	Nos
3	<i>HOXA7</i>	Nos	Nos	Nos	Nos
1	<i>HOXC8</i>	Nos	Nos	Nos	Nos
2	<i>NECAB1</i>	Nos	Nos	Nos	Nos
4	<i>NEU1</i>	Nos	Nos	Nos	Nos
4	<i>NOVA1</i>	Nos	Nos	Nos	Nos
3	<i>NPTN</i>	Nos	Nos	Nos	Nos
2	<i>PLK2</i>	Nos	Nos	Nos	Nos
1	<i>RRAGD</i>	Nos	Nos	Nos	Nos
4	<i>SLC48A1</i>	Nos	Nos	Nos	Nos
4	<i>TFAP2C</i>	Nos	Nos	Nos	Nos
3	<i>TLCD1</i>	Nos	Nos	Nos	Nos
2	<i>TNFAIP8</i>	Nos	Nos	Nos	Nos
5	<i>TRIM71</i>	Nos	Nos	Nos	Nos
5	<i>ZNF253</i>	Nos	Nos	Nos	Nos
2	<i>ZNF791</i>	Nos	Nos	Nos	Nos
4	<i>ZNF83</i>	Nos	Nos	Nos	Nos

Appendix 5 : Non-Significant Genes Identified UALCAN Proteomic Expression Profile

GENE RANK to miRNA	<i>Gene</i>	Comparison Normal V All Samples	Comparison Normal V Grade 3	Comparison Normal V Stage 4	Comparison Normal V Stage 3
5	<i>ANKRD45</i>	Nos	Nos	n/a	Nos
2	<i>CAPZB</i>	Nos	Nos	Nos	Nos
1	<i>CHN1</i>	Nos	Nos	n/a	Nos
3	<i>CST3</i>	Nos	Nos	Nos	Nos
3	<i>NOVA1</i>	Nos	Nos	n/a	Nos
4	<i>TFAP2C</i>	Nos	Nos	N/A	Nos
4	<i>TLCD1</i>	Nos	Nos	N/A	Nos
5	<i>TNRC6A</i>	Nos	Nos	N/A	Nos

Appendix 6 : Search Strings Utilized for OC Gene Association Studies

Database	Search String
Pubmed	("ovarian cancer") AND ("ZNF460" OR "ZNF804B" OR "GCC2" OR "HMGA2" OR "BDNF" OR "BICC1" OR "C17orf62" OR "RRAGD" OR "ISL1" OR "HOXC8" OR "CCL7" OR "NECAB1" OR "PLK2" OR "ARID3B" OR "ARSJ" OR "TNFAIP8" OR "PEX10" OR "ZNF791" OR "TNRC6A" OR "HOXB8" OR "POLR2K" OR "INO80D" OR "AKIRIN1" OR "LIN28B" OR "CRLF3" OR "CHN1" OR "TLCD1" OR "CADM2" OR "NPTN" OR "HOXA7" OR "TAS2R14" OR "DUSP16" OR "NOVA1" OR "TFAP2C" OR "FIGN" OR "NEU1" OR "SLC48A1" OR "ZNF83" OR "CAPZB" OR "NR6A1" OR "RP11-386G21.1" OR "ZNF253" OR "SZRD1" OR "HOXA3" OR "TRIM71" OR "C11orf86" OR "CST3" OR "ANKRD45" OR "PRRX1"). Searching Title/Abstract for this term yielded 149 results spanning over 22 years (2002 – 2024). This search was rerun including only the genes identified as significant in Table 0 12 : ("ovarian cancer") AND ("ZNF791" OR "PRRX1" OR "BICC1" OR "TNFAIP8" OR "GCC2" OR "HMGA2" OR "CRLF3" OR "NPTN" OR "SZRD1" OR "ZNF460" OR "POLR2K" OR "ZNF253").
Pubmed	((("ovarian cancer"[Title/Abstract]) AND ("ZNF791"[Title/Abstract] OR "PRRX1"[Title/Abstract] OR "BICC1"[Title/Abstract] OR "TNFAIP8"[Title/Abstract] OR "GCC2"[Title/Abstract] OR "CRLF3"[Title/Abstract] OR "NPTN"[Title/Abstract] OR "SZRD1"[Title/Abstract] OR "ZNF460"[Title/Abstract] OR "POLR2K"[Title/Abstract] OR "ZNF253"[Title/Abstract]))),

Appendix 7 : Summary of drug dosing concentrations and corresponding plasma levels for cell line treatment models.

Cell line	Drug Dosing Options	Plasma Levels
Carboplatin	Carboplatin 2ug/ml Carboplatin 4ug/ml (McDermott et al)	"After intravenous bolus injection of 375 mg/m ² carboplatin peak-plasma levels of ~39µg/mL are achieved, which drops to 9µg/mL within 2 h" (McDermott et al)
Paclitaxel	Paclitaxel 12ng/ml Paclitaxel 60ng/ml (McDermott et al)	"Pharmacokinetic studies for Paclitaxel dose show peak-plasma concentrations as high as 10,000 ng/mL but drop off quickly after 24 h to 50 ng/mL and below" (McDermott et al)
Olaparib	The human HGSOC cell line resistant to olaparib (PEO1-OR) by constant exposure to gradually increasing concentrations of olaparib by 2-fold (10–80 µM) during culture of cells over the period of 20 days. Primarily used doses of olaparib were based on an IC₅₀ value of olaparib determined from a dose–response curve over 5 days in the parental cell line. Briefly, the cells were exposed to a range of doses of olaparib (0–120 µM or 0–240 µM), and calculated IC₅₀ values were compared between the established and the parental cell line to define the increase in drug resistance PARPi-resistant cell strains (A2780R, SKOV3R and HEYR) were generated by treating parental cells (A2780, SKOV3 and HEY, respectively) with long-term increasing concentrations of olaparib (nearly 1 year; 1–100 µmol) as described in the Results section. https://pubmed.ncbi.nlm.nih.gov/36270832/	"A phase 1 study on Japanese patients found that peak-plasma values for a single dose of 400 mg olaparib was ~7µg/mL, which dropped below 0.1µg/mL after 50 h. For a dose of 400 mg administered twice daily for 15 days, peak-plasma concentrations were found to be similar." (McDermott et al, Yamamoto et al)

Carboplatin

Plasma concentrations drop to 9µg/mL within 2 hours

Carboplatin has a molecular weight of 371.25 ([Carboplatin \(NSC 241240\) Datasheet \(selleckchem.com\)](#))

Using Graphpad ([Molarity Calculator \(graphpad.com\)](#))

3. Molarity from mass & volume

Mass: micrograms 

Formula Weight (daltons):

Volume: milliliter 

Molarity =

Paclitaxel

following 24 hours 50 ng/mL

Molecular Weight of Paclitaxel is 853.91 ([Paclitaxel \(NSC 125973\) Datasheet \(selleckchem.com\)](#))

Using Graphpad

3. Molarity from mass & volume

Mass: micrograms 

Formula Weight (daltons):

Volume: milliliter 

Molarity =

Olaparib

Based on the above, peak plasma concentrations were 7 µg/ml

Olaparib has a molecular weight of 434.46 ([Olaparib \(AZD2281\) Datasheet \(selleckchem.com\)](#))

Using [Molarity Calculator \(graphpad.com\)](#)

3. Molarity from mass & volume

Mass: micrograms ▼

Formula Weight (daltons):

Volume: milliliter ▼

Molarity =

Appendix 8 : Custom designed primer sequence for circRNAs

circRNA	Custom Designed Divergent Primers
Circ400223	FP1 5'- GGG AGC GGC TAG AGC TCT GT -3' FP2 5'- CGA GGG ACC ATT GCA TAG GA -3' RP1 5'- AAA TGG CAG AAT GCG GTC TC -3'
hsa_circRNA_017873	FP1 5'- TTC CAG CAA GTA TCC AAC CA -3' RP1 5'- CCT CTT CGT GGA GTT TCT TCA-3'
hsa_circRNA_0021732	FP1 5'- AAG GTG GAG CAA ACA CAA CC-3' RP1 5'- GAT GCT CAT GGT GAA TGA GG-3'
hsa_circRNA_007919	FP1 5'- GAC AAG ATC ATG GGC AAA GG-3' RP1 5'- AGG GCT TCC AGC TGG TTA AT-3'
hsa_circRNA_003300	FP1 5'- CAT GTA CCA TCA CCA GCA GTG-3' RP1 5'- CAT TGC CAC TTG AAC TCT TCC-3'
hsa_circRNA_008026	FP1 5'- CCT TAT GGA AAG CCA GTG GA -3' RP1 5'- GCA AAT TCT CAG GCT TCA GG -3'
hsa_circRNA_021732	FP1 5'- AAG GTG GAG CAA ACA CAA CC -3' RP1 5'- GAT GCT CAT GGT GAA TGA GG -3'
hsa_circRNA_100059	FP1 5'- ACA ATG CAT GCC CTC GTT AT-3' RP1 5'- TGA TGT GTT GGG ACT TGA GG -3'
hsa_circRNA_102787	FP1 5'- GCT CTC CAA GTT CAG CAT CC -3' RP1 5'- TTA AAC GTG CCA TCA TCC TG -3'
hsa_circRNA_104925	FP1 5'- CAT CCT TAT GCG CGT CAC T -3' RP1 5'- GTC AGG ATC TTC CCG GTT TT -3'
hsa_circRNA_026359	FP1 5'- CCT GGT CCC AAG ACA GTG AG -3' RP1 5'- GGT CTC CAG CAG CTT GTT CT -3'
hsa_circRNA_061800	FP1 5'- CCC AGT GTT TTA GGA GCA TGA-3' RP1 5'- CGG ATC AGC TTC TCA CCT TC -3'
hsa_circRNA_025636	FP1 5'- GGT GGA TGG AGT TCA GAG GA -3' RP1 5'- AGT CTC CGA GCC AGT GAG AA -3'
hsa_circRNA_088194	FP1 5'- CTG CCA CGG AAT ACA CAC TG -3' RP1 5'- TTG TCG AAG ACC CCT TCA TC -3'

Appendix 9 : Dosing schedule and escalation of Carboplatin, Paclitaxel, Olaparib, and combination doublet therapy across OC cell lines

		Sep-23	Oct-23	Nov-23	Dec-23	Jan-24	Feb-24	Mar-24	Apr-24	May-24	Jun-24	Jul-24	Aug-24	Sep-24	Oct-24	Nov-24
COV362	Carboplatin (uM)	6	6	6	6	6	6	8	12	16	20	20				
COV362	Paclitaxel (nM)	5	5	5	5	5	5	10	10	20	20	20				
COV362	Olaparib (uM)	10	10	10	10	10	10	16	24	30	32	40	←			60
COV362	Doublet (uM, nM)	2/ 5	4/ 10	4/ 10	4/ 10	6/ 10	6/ 10	6/ 10	8/ 10	8/ 15	8/ 15	8/ 15	8/ 10	10/ 10	10/ 15	10/ 15
Kuramochi	Carboplatin (uM)	2	2	4	6	4	6	8	20	30	30	30				
Kuramochi	Paclitaxel (nM)	5	5	5	10	5	10	10	15	20	20	20				
Kuramochi	Olaparib (uM)	10	10	10	10	10	10	16	24	32	40	40	40	60	80	100
Kuramochi	Doublet (uM, nM)	2/ 5	4/ 10	4/ 10	4/ 10	6/ 10	6/ 10	6/ 10	8/ 10	8/ 10	8/ 10	8/ 10	8/ 10	10/ 10	10/ 15	10/ 15
Ovsaho	Carboplatin (uM)	2	2	2	2	2	2	6	10	12						
Ovsaho	Paclitaxel (nM)	→				5	5	10	15	15						
Ovsaho	Olaparib (uM)	→				8	16	20	24	40	40	80	80	100	80	100
Ovsaho	Doublet (uM, nM)	→				2/ 5	2/ 5	2/ 5	4/ 5	8/ 10	8/ 10	8/ 10	8/ 10	10/ 10	10/ 15	10/ 15

This table summarises the dosing schedule and escalation of Carboplatin, Paclitaxel, Olaparib, and combination doublet therapy across three OC cell lines: COV362, Kuramochi, and OVSAHO. Values represent the dose (in μM or nM) administered at each monthly timepoint. Green-shaded cells indicate when resistance was established, defined by stable cell growth at the target dose without ongoing cytotoxicity. Red-shaded cells reflect timepoints when resistance had not yet developed, based on viability assays or observed cell death. Arrows denote instances of contamination where an earlier cryopreserved passage was thawed and treatment resumed without altering the dose schedule.

Appendix 10 : STR Profiling results

[illegible]

Appendix 11 : Primers for circRNA resistance models

Condition	Name	Sequence	Scale	Purification
down_COV362- UT_vs_COV362-C_T	hsa_circ_0007008 FP1	TCCTAGCACCCACTCGAGAA	25nm	STD
down_COV362- UT_vs_COV362-C_T	hsa_circ_0007008 RP2	GGCCTGGGTGGCTTTTAGAT	25nm	STD
down_COV362- UT_vs_COV362-C_T	hsa_circ_0085803 FP1	CTCCAGTGTGACCTCCTTGG	25nm	STD
down_COV362- UT_vs_COV362-C_T	hsa_circ_0085803 RP1	GGCGATGGATCCGTATGACA	25nm	STD
down_COV362- UT_vs_COV362-C_T	hsa_circ_0040800 FP1	GCTAACCTGTTACTACTGGGCT	25nm	STD
down_COV362- UT_vs_COV362-C_T	hsa_circ_0040800 RP2	TCACGCTGTAGCAGTTCACG	25nm	STD
down_COV362- UT_vs_COV362-PARPi	hsa_circ_0051239 FP1	ACACGCTGTCCTTACTACTGG	25nm	STD
down_COV362- UT_vs_COV362-PARPi	hsa_circ_0051239 RP1	TCCCACATCCTCGAACAATCC	25nm	STD
down_COV362- UT_vs_COV362-PARPi	hsa_circ_0003258 FP1	GCACAAACAGTTCATGTGCCA	25nm	STD
down_COV362- UT_vs_COV362-PARPi	hsa_circ_0003258 RP1	AAGGAGTGCACCTTGAGTGA	25nm	STD
down_COV362- UT_vs_COV362-PARPi	hsa_circ_0051238 FP1	ACACACACGCTGTCCTTACA	25nm	STD
down_COV362- UT_vs_COV362-PARPi	hsa_circ_0051238 RP1	CGATGGATGCCCTGATACG	25nm	STD
