

PLAT-M8 Prognostic Biomarker, Its Related Genes, and Mechanistic Insights in Ovarian Cancer: A Systematic Review with Bioinformatic Validation

Muhammad Habiburrahman¹, James M Flanagan^{1*}

¹ Division of Cancer, Department of Surgery and Cancer, Imperial College London, London, United Kingdom, W12 0NN

*Address for correspondence:

Prof. James M. Flanagan

Division of Cancer, Department of Surgery and Cancer,

Faculty of Medicine, Imperial College London

4th Floor IRDB, Hammersmith Campus, Du Cane Road, London W12 0NN

Tel: 020 75942127; email: j.flanagan@imperial.ac.uk

Running Title: PLAT-M8 Biomarker and Mechanisms in Ovarian Cancer

Affiliation Details:

Dr. Muhammad Habiburrahman

- **PhD student** at Division of Cancer, Department of Surgery and Cancer, Imperial College London, Hammersmith Campus, London, United Kingdom and **Medical Doctor** at the Faculty of Medicine, Universitas Indonesia, Dr. Cipto Mangunkusumo Hospital, Central Jakarta, DKI Jakarta, Indonesia
- **Email:** h.habiburrahman23@imperial.ac.uk / muhammad.habiburrahman51@ui.ac.id
- **ORCID iD:** <https://orcid.org/0000-0001-6372-8240>
- **Mailing address:** 4th Floor IRDB, Hammersmith Campus of Imperial College London, Du Cane Road, London, United Kingdom, W12 0NN

Dr. James M. Flanagan

- **Professor of Informatics, Director** of the MRes Cancer Biology, and **Head of Translational Epigenomics Research Group** at Division of Cancer, Department of Surgery and Cancer, Imperial College London, Hammersmith Campus, London, United Kingdom.
- **Email:** j.flanagan@imperial.ac.uk
- **ORCID iD:** <https://orcid.org/0000-0003-4955-1383>

- **Mailing address:** 1st Floor IRDB, Hammersmith Campus of Imperial College London, Du Cane Road, London, United Kingdom, W12 0NN

Details of Words/ Characters Count:

Title Words/ Characters	: 16 words/ 114 characters
Summary/Abstract	: 120 words
Keywords	: 5 words
Main Article + Figure Legends	: 6,774 words
Number of Figures	: 6 figures
Number of Tables	: 1 table
Short Titles	: 7 words/ 50 characters
Supplementary Materials	
• STAR☆Method	: 1 File
• Supplementary Tables	: 9 Tables
• Supplementary Figures	: 4 Figures

Authors' Contributions:

- **Conceptualisation:** MH, JMF,
- **Data curation:** MH
- **Formal Analysis:** MH, JMF
- **Funding Acquisition:** JMF
- **Investigation:** MH
- **Methodology:** MH, JMF
- **Project Administration:** MH, JMF
- **Resources:** MH, JMF
- **Software:** MH
- **Supervision:** JMF
- **Validation:** JMF
- **Visualisation:** MH
- **Writing – draft preparation:** MH, JMF
- **Writing – review & editing** MH, JMF

JMF served as the principal investigator for this study, secured funding, and made the decision to publish. As the guarantor, JMF assumed full responsibility for the work. MH and JMF conceptualised the research, contributed to the analysis, conducted the investigation, designed the methodology, managed project administration, provided resources, did literature review, drafted the paper, and reviewed and edited the manuscript to the manuscript. MH curated the data, did investigation, utilised software, and created visualisations of the study findings. JMF validated all evidence analyses and thoroughly supervised the study process. All mentioned authors critically revised the manuscript for important intellectual content and approved the final version for publication.

Acknowledgments

The first author would like to acknowledge a scholarship from *Beasiswa LPDP* (the Indonesian Endowment Fund for Education) from the Ministry of Finance of the Republic of Indonesia, which provided support to pursue a PhD at Imperial College London under contract number 20230722299954. The authors acknowledge additional support from the Cancer Research UK Imperial Centre, the National Institute for Health Research Imperial Biomedical Research Centre and the Ovarian Cancer Action Research Centre.

Financial disclosure statement

This study did not receive any grant funding.

Declaration of Interests statement

The authors declare that they have no competing interests.

Ethics statement

Not applicable

Data sharing statement (availability of data and material)

All relevant data and materials supporting the findings of this study are available upon request from the corresponding author.

Figure guidelines

We confirm that our figures have complied with the journal image preparation guidelines. Also, there is no image manipulation in this study.

SUMMARY

Ovarian cancer (OC) is a leading cause of gynecologic cancer-related death, necessitating reliable prognostic biomarkers. PLAT-M8 has emerged as a promising DNA-methylation biomarker influencing key genetic pathways in relapsed OC, though its role in primary OC is less understood. A systematic review of preclinical and clinical studies (2008–2023) was conducted to explore PLAT-M8's prognostic potential in OC, resulting in 27 studies being reviewed. Bioinformatics tools (KM-plotter, TNMplot, Cancer Hallmark) validated the clinical relevance of PLAT-M8 by analysing overall survival, progression-free survival, gene correlations, and tumour vs. normal tissue. Subgroup analyses considered treatment profile, surgery, stage, and histological type. Significant correlations between PLAT-M8 methylation and gene expression were found, underscoring its prognostic value in OC and potential to guide personalised treatment.

HIGHLIGHT:

- **Innovative Prognostic Biomarker:** This review identifies PLAT-M8 as a novel prognostic biomarker in OC, offering new avenues for early prognosis and personalised treatment strategies.
- **Gene Expression Correlations:** We highlight key genes associated with PLAT-M8 methylation, elucidating their roles in tumorigenesis, chemoresistance, and patient survival outcomes.
- **Epigenetic Mechanisms:** Analysis reveals how hypermethylation and hypomethylation of PLAT-M8 CpG sites influence gene expression, providing insights into the underlying epigenetic regulatory mechanisms.
- **Bioinformatics Validation:** Utilising bioinformatics, we validate the clinical relevance of PLAT-M8 and its related genes, ensuring robust and reproducible findings.
- **Personalised Medicine Implications:** Our findings underscore the potential of PLAT-M8 and its associated genes to refine personalised medicine approaches, enhancing treatment efficacy for both primary and relapsed OC patients.

INTRODUCTION

Ovarian cancer (OC) is a significant health issue, particularly in high-income countries [1]. The standard treatment for the majority of OC patients consists of debulking surgery followed by chemotherapy employing platinum and paclitaxel. Although approximately 75% of patients initially exhibit a positive response to platinum, many ultimately experience a relapse due to chemoresistance. This results in treatment failure and is responsible for more than 90% of fatalities [2]. Chemoresistance in OC involves multigene expression regulated by factors such as DNA methylation [3], histone modification [4], and non-coding RNA [5]. Exploring

the interplay between these epigenetic factors and their impact on transcriptomics offers the potential for identifying markers of chemoresistance, relapse, and clinical outcomes [6].

Flanagan et al.[3] discovered an epigenetic signature called PLAT-M8, which consists of eight CpGs associated with DNA methylation and predicts OC prognosis at relapse after platinum treatment. These CpG sites include: cg05529343 (*ZNF385D*), cg12992827 (*ZPLD1*), cg16172923 (*MAD1L1*), cg07960624 (*SAMD12*), cg25953130 (*ARID5B*), cg13691961 (*DUSP6*), cg01692018 (*PPP2R5E*), and cg07573872 (*SBNO2*). However, the molecular mechanism of how they influence tumorigenesis and chemoresistance in OC remains unclear. Moreover, the effect of methylation on PLAT-M8 expression is more complex than the typical inverse relationship found in CpG sites of gene promoters [7,8].

This systematic review aims to address these gaps by investigating the PLAT-M8 epigenetic signature and its association with methylation, gene expression, and mechanistic insights. By examining existing literature and emerging findings, the review seeks to understand how the eight CpGs within PLAT-M8 contribute to tumorigenesis, chemoresistance, and relapse. Additionally, the review aims to bioinformatically validate gene expression related to PLAT-M8 and its correlations with prognostic factors in primary OC, hypothesising that PLAT-M8 gene expression may influence prognosis before relapse, while methylation plays a more significant role after relapse.

This article followed PRISMA guidelines (**Figure 1A**), using a descriptive qualitative approach to investigate the role of PLAT-M8 in OC [9]. The literature search was conducted across multiple databases, including PubMed, Proquest, and Scopus, focusing on specific genes and their aliases. All search queries, keywords, selections, and results are detailed in **Tables S1** and **S2**. Studies were selected based on defined eligibility criteria, including clinical relevance and study design. Quality assessment was performed using Cochrane Review tools (**Table S3**) [10] and the Biomarker Toolkit Assessment (**Table S4**) [11], with data extracted and organised into relevant categories. For bioinformatic validation, various web-based tools were used to analyse PLAT-M8 gene expression in OC tissues. This included differential gene expression comparison using TNMplot [12], cancer hallmark summaries from the Cancer Hallmark tool [13], and prognostic assessments using Kaplan-Meier plotter for overall (OS) and progression-free survival (PFS) [14]. Detailed methodology is available in the **STAR★Method**.

<Figure1>

RESULTS

Selection process and characteristics of study

The search process yielded 1,549 articles. After removing 24 duplicates and screening 1,525 titles and abstracts, 55 articles met the inclusion criteria. Of these, 37 were excluded for irrelevance, secondary research, or mislabeled genes. An additional 9 articles were identified through manual search, resulting in a total of 27 articles for detailed review [15-41]. Study characteristics (**Table 1**) show that one of these 8 genes was first studied as early as 2008, with an increasing trend observed until 2023. Most studies originated from the US (30%) [18,28,29,31,33,34,40] and China (26%) [16,24,25,30,32,37,42] (**Figure S1**). Currently, most research on these eight genes is preclinical [16,21-24,26-35,37,39,40], highlighting the need for clinical validation.

<Table1>

Reliability and quality assessment

As shown in **Table 1** and **Figure S1**, 52% of studies had high RoB and 48% had moderate RoB. The low scores result from our adherence to the highest standards of assessment for interventional studies, which are considered the gold standard for critical appraisal [10]. Despite perceived quality issues and the absence of randomisation and blinding, which contribute to a higher risk of bias, these studies remain valuable for addressing PICO questions. They also make significant contributions to the systematic review, primarily because most mechanistic insights are derived from preclinical research. In terms of quality assessment, we used the Biomarker Toolkit Assessment, which compiles information from all assessment tools for critically appraising biomarkers in research [11]. Our assessment revealed that 56% of studies had low quality for clinical biomarker application, 37% were of moderate quality, and only 7% were deemed high quality for clinical use.

Study design and outcomes measurement

Among the 27 reviewed studies, there were nine different study designs, with many studies featuring multiple designs as defined in our consensus (**Table S5**). Most studies were transcriptomics (81%) [15,16,20,22-24,26-41], followed by experimental *in vitro* (63%) [15,21,22,25-36,39,40], and bioinformatics (52%) [15,16,22-24,30,31,33-35,37,38,41]. Only 33% were clinical observational studies (cohort and cross-sectional) [15,17-20,25,36,38,41]. Studies primarily investigated mRNA (70%) [18,20-23,25-37,41], with some focusing on protein expression and miRNA. The most frequently used samples in OC studies were tumour-derived DNA, blood-derived DNA, cancer cell lines, and cancer tissues. Sample sizes varied widely, from a minimum of 28

(Chinese patients with OC) [24] to a maximum of 2,600 (FOCI collaboration cases) [18], depending on the type of sample and study. In terms of blood samples, the smallest group consists of 6 healthy controls for plasma miRNAs [38], and the largest group comprises 247 peripheral blood DNA samples where this PLAT-M8 was discovered [15]. Tumour tissue samples ranged from 13 (primary epithelial OC tissues) [20] to 1,000 (invasive primary EOC cases) [18], with 46 for tumour DNA samples during the discovery of PLAT-M8 [15]. The top OC cell lines studied were SKOV3 (7 mentions) [26,28-30,35,36,39], A2780 (4 mentions) [15,26,32,33], OVCAR8 (4 mentions) [28,30,33,39], OVCAR3 (3 mentions) [26,30,33], and IGROV-1 (2 mentions) [22,27]. Outcome measurements were diverse (**Table S6**), with *DUSP6* (n=10) [15,26-34], *PPP2R5E* (n=5) [36-40], *ARID5B* (n=5) [21-25], and *MAD1L1* (n=4) [17-20] being the most frequently studied genes, underscoring their roles as prognostic factors and predictors of chemotherapy response. Meanwhile, *ZNF385D* [15,16] and *SBNO2* [15,41], *ZPLD1* [15], and *SAMD12* [15] have been studied to a limited extent. Overall, PLAT-M8-related genes are crucial for understanding tumorigenesis, disease progression, and treatment response (**Figure S1**).

Bioinformatics validation

We validated the PLAT-M8 genes using web-based tools to compare their expression in normal versus cancer tissues. The violin plot (**Figure 2A**) shows a lower expression of *ZNF385D* and *ZPLD1* in tumours, slightly higher *MAD1L1* expression, and significantly higher *SAMD12* and *DUSP6* levels in tumour tissue. *ARID5B*, *SBNO2*, and *PPP2R5E* show similar expression in both normal and tumour tissues. The targetgram (**Figure 2B**) reveals higher mean values for *SBNO2*, *ARID5B*, and *PPP2R5E* in normal tissues, while tumour tissues show higher values for *SBNO2*, *ARID5B*, *DUSP6*, *PPP2R5E*, *MAD1L1*, and *SAMD12*. The gene signature analysis (**Figure 2C**) indicates a change of 1.33-fold for tumour expression. Pathway analyses show that PLAT-M8 genes are involved in protein dephosphorylation (**Figure 2D**). They are also biologically associated with sequence-specific DNA binding in terms (**Figure 2E**) and are primarily localised in the nucleus and intracellular organelles (**Figure 2F**). Furthermore, significant disease mechanisms include replicative immortality and reprogramming energy of metabolism ($p < 0.001$) (**Figure 2G**). Notably, gene-specific associations include *PPP2R5E* and *DUSP6* with proliferative signalling, *MAD1L1* with replicative immortality, and *PPP2R5E* with multiple mechanisms including resisting cell death and reprogramming of energy metabolism. Moreover, the correlation plot (**Figure 2H**) shows weak correlations between some gene pairs, such as *SBNO2* with *ZPLD1* and *MAD1L1*; *SAMD12* with *ARID5B* and *DUSP6*; *ARID5B* with *DUSP6* and *PPP2R5E*; *DUSP6* with *PPP2R5E*; and *PPP2R5E* with *SBNO2*.

<Figure2>

The prognostic performance of PLAT-M8 genes for OS and PFS was validated using mRNA microarray datasets (**Figures 3A and 3B**). Among overall cases, the PLAT-M8 gene signature significantly predicted OS (n=655, hazard ratio/HR 1.38, 95%CI: 1.13-1.70, p=0.0016), with key predictors being upregulation of *ZNF385D* and *ARID5B*, and downregulation of *DUSP6*. For PFS in 614 cases, the signature also significantly predicted outcomes (n=614, HR 1.38, 95%CI: 1.14-1.66, p=0.0008), with *DUSP6* being the primary gene associated with the prediction. Subgroup analyses demonstrated consistent OS predictive value across all cases and the platinum-containing treatment subgroup, although slightly lower in the latter (HR 1.34, 95%CI: 1.08-1.70, p=0.013). Key genes for OS included increased *ZNF385D* and decreased *SAMD12* and *DUSP6* (**Figure 3C**). For PFS, predictions were slightly lower in the platinum subgroup (HR 1.27, 95%CI: 1.05-1.55, p=0.014), with significant genes being upregulated *ZNF385D* and *MAD1L1*, and downregulated *SAMD12* (**Figure 3D**). These findings emphasize PLAT-M8's relevance, particularly about platinum resistance in relapsed OC.

<Figure3>

PLAT-M8 genes are also linked to unfavourable clinical outcomes, including relapse risk, with a notable association in advanced stages (HR 1.41, 95%CI: 1.12-1.77, p=0.0031) in OS analysis. They did not significantly impact prognosis in debulking surgery cases, though individual genes like increased *MAD1L1* and *ARID5B*, and decreased *SAMD12*, can predict OS. For PFS, PLAT-M8 genes showed predictive values in both suboptimal debulking (HR 1.32, 95%CI: 1.00-1.73, p=0.046) and advanced stages (HR 1.36, 95%CI: 1.12-1.65, p=0.00019). In these cases, *ZNF385D*, *MAD1L1*, and *ARID5B* were upregulated, while *SAMD12* and *DUSP6* were downregulated.

DISCUSSION

PLAT-M8, a blood-based DNA methylation biomarker, was associated with post-relapse survival. However, it was not associated with survival when measured at the time of primary diagnosis [15]. Therefore, this systematic review and bioinformatics validation of tumour biopsy datasets aims to investigate whether PLAT-M8 gene expression can be used as a prognostic indicator at the time of primary diagnosis. Although blood data was not available for validation, we hypothesise similar gene expression patterns between tumour biopsies and blood, as observed in methylation patterns [15]. Additionally, we reviewed the impact of platinum adduct on methylation and gene expression patterns in OC in relation to PLAT-M8, as well as the role of PLAT-M8-related gene mechanisms in driving tumorigenesis, progression, metastasis, and chemoresistance.

Reflecting platinum-based chemotherapy: methylation and its effect on gene expression

The use of PLAT-M8 as a methylation biomarker is based on the idea that platinum treatment causes abnormal methylation in OC tissues, detectable in blood, making it logical to measure methylation during relapse. **Figure 4** summarises how platinum adducts can lead to abnormal methylation. These adducts primarily occur within and between DNA strands, resulting in DNA damage [43]. As a consequence, cell structure is disrupted, cell division is inhibited, and transcription and DNA synthesis are blocked [44]. It should be noted that platinum adducts can induce various types of DNA damage [45]. The response to platinum adducts in OC cells hinges on mismatch repair (MMR) efficiency [15,46]. Effective MMR stalls replication at adduct sites, causing stress [15,43,47] and triggering apoptosis without repair, which may enhance chemotherapy sensitivity in OC patients [15,48]. Replication stress can alter the epigenetic profile, including DNA methylation. We postulate that hypermethylation in chemo-sensitive patients silences oncogenes, tumour-driver genes, and drug-resistance genes, following the typical inverse correlation between methylation and gene expression. Replication stress can disrupt the epigenetic profile, including DNA methylation. We propose that hypermethylation in chemo-sensitive patients silences oncogenes, tumour-driver genes, and drug-resistance genes due to the inverse relationship between methylation and gene expression. However, hypermethylation may leave gene expression unaffected or even increase it, as seen with tumour suppressor genes (TSGs), potentially due to inadequate RNA Pol II recruitment [49]. This complicates the idea that DNA methylation always suppresses gene expression. Similar results in prostate cancer show that hypermethylation can both downregulate and upregulate gene expression [50], depending on factors such as tissue type [51], gene location (promoter, intron, or exon), site functionality, regulatory roles [52], and transcription factor (TF) interactions [8,53].

<Figure4>

According to Smith et al. [8], epigenetic regulation is complex and influenced by developmental, environmental, and genetic factors, as well as biases in defining gene regions, methylation cutoffs, and technical variability. Based on this evidence, we hypothesise that hypermethylation from platinum exposure may activate TSGs, playing a role in preventing resistance and tumorigenesis while repressing oncogenes. Most research focuses on promoter methylation, but significant changes are also observed in non-promoter regions, particularly downstream of the transcription start site, such as enhancers, with positive correlations in cancers [54,55]. The PLAT-M8 discovery study focused on gene body regions (in exons or introns) instead of promoters [15]. Even

if PLAT-M8 targets promoter methylation, which typically represses oncogenes, promoter methylation can still increase gene expression by maintaining genomic stability [56,57], modulating histone methylation, and regulating splicing [58,59]. We suggest TSG activation may occur through the inhibition of repressor or insulator binding and activation via alternative promoters [8]. Gene silencing typically occurs through promoter methylation, which disrupts TF binding and recruits chromatin remodelers, leading to closed heterochromatin that blocks transcription [8,60]. Although some TFs can bind methylated CpGs [53,61], sparse promoter methylation may hinder transcription, though enhancers can still activate the gene [62-64]. There is also strong evidence that adjacent mCpG site methylation in regulatory regions is correlated. These regions often become hypermethylated to maintain inactivity when inactive in other cell types [65,66].

On the other hand, the role of gene body methylation in gene expression is not well understood. It is proposed to positively correlate with gene expression and potentially link distal regions with enhancers [15,53,67]. Gene body methylation can suppress gene expression by promoting chromatin densification and interacting with functional elements like alternative promoters, enhancers, TF binding sites, and repetitive elements [67,68]. However, it also positively influences transcription by preventing spurious transcription, regulating alternative splicing, and ensuring proper splicing, translation, and stable transcription. Additionally, gene body methylation both regulates and is regulated by active transcription [67]. Evidence shows that gene body methylation can alter TF binding affinity [61]. Methylated intron regions, such as those containing TF motifs *paired box 5* and 6 (*PAX5/6*), were found in tumours [69]. Lai et al. [70] reported that hypermethylation of CpG islands in the first intron of the *B-cell lymphoma 6* (*BCL6*) correlates with high *BCL6* expression. Similarly, hypermethylation of specific introns like *early growth response 2* (*EGR2*) intron 1 can activate gene expression [71]. In exons, hypermethylation, especially near the start and termination sites, is linked to increased exon inclusion and gene expression [67,72]. This occurs through two mechanisms: the kinetic model, where hypermethylation slows RNA Pol II, enhancing exon recognition, and the recruitment model, where it attracts splicing factors. Hypomethylation, on the other hand, accelerates transcription, leading to exon skipping and reduced expression [67,72]. In tumours, hypermethylation of CpG islands in exons, such as those in the *HOX transcript antisense RNA* (*HOTAIR*), enhances transcription and promotes tumour development [73]. Neri et al. [74] found that gene body methylation is crucial for regulating RNA transcription from exons and maintaining transcriptional fidelity by blocking abnormal intragenic transcription. Overall, while promoter methylation is well-studied, gene body methylation—particularly at PLAT-M8 CpG sites in exons and introns—plays a crucial role in regulating gene expression and tumour development.

In the PLAT-M8 framework (**Figure 4**), hypomethylation is more common in chemo-resistant patients. Poor MMR capacity causes replication stalling at platinum-DNA adducts, disrupting normal methylation and leading to hypomethylation [15,75]. This stalling increases drug detoxification and impacts DNA methylation by altering the cellular environment. The resulting transcriptional reprogramming, changes in regulatory proteins, and failures in stress-response pathways contribute to hypomethylation and increased expression of resistance-related genes [15,76,77]. Replication stalling promotes translesion synthesis [78,79], allowing malignant cells to bypass platinum-DNA damage, leading to chemoresistance and sustained hypomethylation. This hypomethylation affects TF binding and modifies gene expression through epigenetic reprogramming [80]. Cancer DNA hypomethylation can lead to global hypomethylation and oncogene overexpression, causing DNA instability, increased mutations, and altered gene expression [81]. For example, hypomethylation of *cyclin-dependent kinase 2* (*Cdk2*) is linked to increased expression and cancer progression in OC [82]. Other genes, like *ATP-binding cassette sub-family B member 1* and *sub-family G member 2* (*ABCB1* [83] and *ABCG2* [84]), and *phosphoinositide-3-kinase regulatory subunit 3* (*PIK3R3*) [85], are hypomethylated and contribute to chemoresistance. Additionally, hypomethylation of oncogene promoters, including *solute carrier family 6 member 12* (*SLC6A12*) [86], *cancer/testis antigen 45* (*CT45*) [87], *carbonic anhydrase 9* (*CA9*) [88], and *histone cluster 1 H2BN* (*HIST1H2BN*) [89], is associated with poor survival [90]. Unlike hypermethylation, hypomethylation rarely causes transcriptional repression [8], but targeting it could improve treatment, as shown with decitabine in platinum-resistant OC patients [91].

Despite the paradox between methylation and gene expression, unique patterns emerged in PLAT-M8 genes. Higher expression of five genes (*ZNF385D*, *ZPLD1*, *MAD1L1*, *ARID5B*, *PPP2R5E*) is associated with poorer prognosis, tumorigenesis, metastasis, and chemoresistance. Hypermethylation is thought to silence these genes, which could improve outcomes and treatment sensitivity. Conversely, lower expression of the three other genes (*SAMD12*, *DUSP6*, *SBNO2*), thought to act as TSGs, is linked to poorer survival. Hypomethylation of these genes may improve outcomes. However, the combined effect of the eight genes shows that their increased expression correlates with worse survival rates, likely due to PLAT-M8's hypomethylation status, which we previously identified as a Class 1 biomarker [15].

PLAT-M8 gene mechanisms in tumorigenesis, progression, metastasis, and chemoresistance

Over the past 16 years, research has extensively examined *DUSP6*, *PPP2R5E*, *ARID5B*, and *MAD1L1* within the PLAT-M8 genes in ovarian cancer, with *ZNF385D*, *SBNO2*, *ZPLD1*, and *SAMD12* also studied. However, the impact of methylation on gene expression in ovarian carcinogenesis remains underexplored. Our review

indicates significant bias and limited biomarker performance assessment in existing studies, underscoring the need for more clinical validation (**Table 1**). The comprehensive signalling pathways of these eight genes, relevant to tumorigenesis and disease progression, are summarised in **Figure 5**.

<Figure5>

DUSP6 (*MKP3*) can act as either an oncogene or tumour suppressor, depending on its expression. It regulates the extracellular signal-regulated kinase (ERK) signalling pathway, and its loss promotes tumour growth and spread [26,27]. Our analysis links lower *DUSP6* expression to poorer survival and increased tumour progression, although some studies report higher [28], especially in chemotherapy-resistant tissues [30]. *DUSP6* negatively regulates the Mitogen-Activated Protein Kinase (MAPK)/ERK pathway [92], and reduced levels lead to increased ERK1/2 phosphorylation, boosting cell proliferation and differentiation [33]. Human epididymis protein 4 (HE4), an established OC biomarker, affects *DUSP6* expression. Higher HE4 levels can increase *DUSP6* production in some cells and alter its expression in others, suggesting HE4 promotes disease progression [28]. The stress hormone norepinephrine and transforming growth factor-beta 1 (TGFβ1) also lower *DUSP6* levels in ovarian and fallopian tube cells, indicating *DUSP6*'s role in stress responses and tumour adaptation [31,35]. Low *DUSP6* levels reduce interactions with tumour suppressors like p53, affecting cell fate. Under genotoxic stress, p53 is activated for cell cycle arrest and death [93,94], but low *DUSP6* levels increase ERK and MAPKs pathway activation [95], promoting cell survival, tumorigenesis, and inhibiting apoptosis [96,97]. This downregulation contributes to cytoskeleton rearrangement, epithelial-to-mesenchymal transition (EMT), and enhanced cell migration and invasion. Reduced *DUSP6* also lowers Bcl-2-interacting mediator of cell death, extra-long (Bim-EL), increases *VAV3* proto-oncogene, and promotes changes through snail family transcriptional repressor (*SNAI1/2*) and zinc finger E-box binding homeobox 1 (*ZEB1*) complexes [95], leading to overactivation of fibroblast growth factor (FGF) [95,98], and further facilitating EMT and cancer cell invasion [96,99].

DUSP6 was also found to be underexpressed in cisplatin- and oxaliplatin-resistant cell lines, leading to increased phosphor-ERK1/2 and drug resistance [26,27]. By regulating ERK1/2 phosphorylation, *DUSP6* influences chemoresistance and the efficacy of targeted treatments [33]. It also affects human epidermal growth factor receptor 2 (HER2) inhibitor response in breast cancer [100] and trametinib sensitivity in low-grade serous OC (LGSOC) [101]. Upregulation of *DUSP6* enhances trametinib sensitivity, while its depletion reduces it [32]. Reduced *DUSP6* impairs ERK1/2 dephosphorylation, increasing chemoresistance and

worsening survival. Reintroducing *DUSP6* restores drug sensitivity and reduces ERK activity, indicating its potential to overcome resistance and improve treatment outcomes [32]. In another study, recombinant HE4 treatment also elevated *DUSP6* in OVCAR8 and SKOV3 cells, correlating with HE4 levels in EOC tissues. Co-treatment with a *DUSP6* inhibitor and chemotherapy reduced *erythroblastic leukaemia viral oncogene homolog 3 (ERBB3)* mRNA levels more effectively than chemotherapy alone [28]. Additionally, HE4 can boost *DUSP6* in immune cells, reducing their anti-cancer effectiveness and contributing to immune suppression [29]. In high-grade serous OC (HGSOC), *DUSP6* is crucial for chemotherapy response, affecting sensitivity to the Poly(ADP-ribose) polymerase inhibitor (PARPi) olaparib. The *DUSP6/1* inhibitor, *DUSPi*, showed strong antitumour activity in both olaparib-sensitive and -resistant HGSOC models, highlighting *DUSP6*'s therapeutic potential [34]. Overall, *DUSP6* acts as both an oncogene and a tumour suppressor, making it a potential therapeutic target in OC due to its role in regulating the MAPK/ERK pathway and chemotherapy resistance.

The second most studied PLAT-M8 gene, *PPP2R5E*, is a "B type" subunit of the protein phosphatase 2A (PP2A) complex [102], crucial in OC for regulating the cell cycle, growth, differentiation, and apoptosis [36,38]. Dysregulation of *PPP2R5E* leads to uncontrolled cell growth and survival, contributing to cancer development [36]. *PPP2R5E* is linked to EMT, enhancing cancer cell mobility, invasion, and metastasis, making it a potential biomarker or treatment target in OC [39]. It is also implicated in major depressive disorder (MDD) in OC patients, highlighting its broad significance in the miRNA-mRNA network [37]. Increased *PPP2R5E*, often due to *BRCA1* mutation and hypoxia, inhibits MEK1/ERK via its PP2A subunit, promoting cell replication and mitogenic signalling [103]. *PPP2R5E* also suppresses mechanistic target of rapamycin (mTOR), enhancing Treg cell function and immune evasion while increasing interleukin-2 receptor/signal transducer and activator of transcription 5/forkhead box protein P3 (IL-2R/STAT5/Foxp3) levels, which weakens the antitumour response [102,104]. It promotes oncoprotein translation by inhibiting eukaryotic translation initiation factor 4E/mitogen-activated protein kinase interacting protein kinase (eIF4E/Mnk), activates the wingless-related integration site (Wnt) pathway by inhibiting T-Cell Factor (TCF)/ β -catenin [105], and enhances cell migration by inhibiting receptor for activated C kinase 1 (RACK1) [104]. Under hypoxia, *PPP2R5E* elevates hypoxia-inducible factor alpha (HIF- α) expression, boosting vascular endothelial growth factor (VEGF) levels, angiogenesis, and metastasis [104]. It also inhibits ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), and checkpoint kinase 1/2 (CHK1/2) pathways, strengthening DNA damage response and chemoresistance [104,106,107]. *PPP2R5E* also increases p21 [108], Rb1 [104], and Gsk3b [104], sustaining cell proliferation, stemness, and resistance.

The third gene, *ARID5B*, significantly influences OC tumorigenesis, progression, and metastasis. *ARID5B*, widely expressed across tissues, forms a complex with plant homeodomain finger 2 (PHF2), influencing chromatin remodeling and gene expression through lysine demethylation [21]. This interaction affects tumour biology, with *ARID5B* expression varying between OC and normal tissues, suggesting its role in cancer [21]. Its downregulation after EPZ-5676 treatment indicates a role in promoting tumour growth and metastasis. *ARID5B* also interacts with *TEAD4* to enhance cell proliferation and migration, and its expression, influenced by copy number changes, makes it a potential biomarker for immune checkpoint therapy in ovarian serous cystadenocarcinoma [24]. Additionally, *ARID5B* facilitates sorbin and SH3 domain containing 2 (SORBS2) histone demethylation, affecting EMT and tumour progression [25]. In acute lymphoblastic leukemia (ALL), *ARID5B* is upregulated and forms a positive autoregulatory loop with Myb and partners like GATA-binding protein-3 (GATA3), runt-related TF 1 (RUNX1), and T-cell ALL 1 (TAL1), enhancing oncoprotein transcription and leading to T-cell dysfunction, increased proliferation, genomic instability, and tumorigenesis [109,110]. As a transcriptional controller, *ARID5B* influences oncoprotein production, cell survival, and T-cell differentiation, potentially impairing T-cell function and reducing immunotherapy effectiveness [109,110]. Therefore, *ARID5B* is a potential biomarker for immune checkpoint inhibitor therapy in OC. Overall, *ARID5B*'s dysregulation in OC contributes to uncontrolled cell proliferation, EMT, and chemoresistance, making it a promising biomarker and therapeutic target.

MAD1L1 is deeply involved in tumour development and metastasis, especially in OC. Variants (e.g., 1673 G-A polymorphism) affect the mitotic spindle assembly checkpoint (SAC), impacting cell division [17]. While allele frequencies are balanced in healthy individuals, cancer patients show a shift, with wild-type genotypes prevalent in early-stage tumours and non-wild-type in advanced cases [19]. This variation affects mitosis, genomic stability, and treatment effectiveness. Upregulated *MAD1L1* in OC increases MAD1 protein levels, which interacts with kinetochores and the mitotic checkpoint complex (MCC) to delay mitosis until chromosomes are properly aligned. Dysfunctional MAD1 leads to chromosomal instability, checkpoint failure, and tumour progression, particularly in advanced stages. The MCC, including BUB1-related kinase 1 (BUBR1), BUB1-binding protein 3 (BUB3), and cell division cycle 20 (CDC20), inhibits the anaphase-promoting complex (APC) until chromosomes are aligned [19,111]. MAD1 dysfunction disrupts microtubules and MCC function, leading to instability, aneuploidy, and cancer progression [19,111]. The 1673 G-A polymorphism of *MAD1L1* impacts treatment outcomes: the GG genotype, associated with higher mitotic arrest, improves sensitivity and response, while the AA genotype, linked to impaired mitotic spindle assembly, leads to increased chemoresistance and poor outcomes [17]. *MAD1L1* also affects response to microtubule-targeting agents;

reduced MAD2 levels are linked to poor outcomes and chemoresistance [19]. Overall, *MAD1L1* influences OC by affecting mitotic spindle assembly and genomic stability, with genetic variations and upregulation contributing to chromosomal instability, disease progression, and treatment resistance.

ZNF385D interacts with m6A regulators such as insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) and zinc finger CCCH-type containing 13 (ZC3H13), affecting epigenetic regulation and contributing to tumorigenesis. It is often overexpressed in cancers due to stress-induced signalling that boosts myelocytomatosis viral oncogene homolog (cMyc), SRY-box TF 4 (SOX4), matrix metalloproteinase (MMP), and nuclear factor kappa beta (NFK β) expression, or through epigenetic modifications by DNA-damaging drugs like platinum [112]. Activated *ZNF385D*, through phosphorylation or acetylation, alters actin filaments at focal adhesion sites [113], promoting EMT, β -catenin activity, cell self-renewal, migration, and invasion, thus aiding metastasis [112,114]. Additionally, phosphorylated *ZNF385D* targets genes related to cellular proliferation and differentiation [112,114,115]. Altogether, *ZNF385D* promotes OC progression by enhancing epigenetic regulation, EMT, and metastasis through its interactions with m6A regulators and overexpression due to stress and DNA damage.

SBNO2, though not widely studied in OC, influences tumorigenesis, progression, and metastasis by regulating immune responses and inflammation, potentially affecting tumour-infiltrating lymphocyte (TIL) counts and the tumour microenvironment [41]. In haematological malignancies, oxidative stress and STAT mutations via the Janus kinase (JAK) pathway increase *SBNO2* to control inflammation [116]. However, epigenetic modifications can reduce *SBNO2*, leading to dysregulated inflammation, elevated IL-6, and cancer progression [117]. Overall, *SBNO2* regulates immune responses and inflammation in OC, affecting the tumour microenvironment and survival, making it a potential therapeutic target.

ZPLD1 and *SAMD12* are not extensively studied in OC. Flanagan et al. [15] examined their methylation, with broader research suggesting *ZPLD1* overexpression may drive EMT and metastasis through mesenchymal engulfment [118], while reduced *SAMD12* expression, possibly acting as a tumour suppressor by inhibiting the MAPK/ERK pathway, is linked to poor survival and increased cell proliferation [137]. To validate the mechanisms of all eight genes, bioinformatics analysis of the PLAT-M8 signature was conducted. This revealed key biological pathways, such as protein dephosphorylation and sequence-specific DNA binding, primarily occurring in the nucleus and intracellular organelles. These pathways contribute to tumorigenesis, disease progression, metastasis, and chemoresistance by enabling replicative immortality and reprogramming energy metabolism, as shown in **Figures 2 and 5**.

PLAT-M8 as a prognostic biomarker

Few studies have examined the prognostic value of PLAT-M8 genes in OC. This review confirms that both PLAT-M8 methylation in blood at relapse and gene expression from tumour biopsies offer robust prognostic insights. Methylation is significant for relapsed patients, while gene expression provides valuable information, especially in primary cases. There are limited studies investigating PLAT-M8 as a prognostic factor in primary cases. Among these, *ARID5B* [21-24] and *MAD1L1* [17-19] are the most studied, according to the systematic review. However, for relapsed patients, both univariate and multivariate Cox regression identified eight significant CpG sites that correlate with PLAT-M8 genes and could serve as prognostic factors for survival after relapse. Assuming that Class 1 (hypomethylated) PLAT-M8, as indicated by Flanagan et al. [15], reflects higher gene expression activity, it correlates with poorer survival and serves as a useful marker for disease progression.

The most studied prognostic gene for OC is *ARID5B*, notably identified through methylation at CpG site cg25953130. Increased methylation at this site correlates with better survival outcomes, positioning *ARID5B* as a potential prognostic biomarker. Its methylation levels offer insights into disease prognosis and treatment response, particularly for primary chemotherapy [15]. Additionally, *ARID5B*'s inclusion in a prognostic model with a coefficient of 0.334 underscores its importance in predicting patient outcomes and guiding treatment decisions [23]. However, Yan et al. [24] found that high *ARID5B* expression correlates with better outcomes, indicating variability in its prognostic value. *ARID5B* is a crucial prognostic factor in OC, affecting tumour behaviour and patient outcomes through its roles in methylation and progression. Its potential as a biomarker and therapeutic target merits further research. The second gene, *MAD1L1*, significantly impacts OC prognosis by affecting chromosomal stability and treatment response. The 1673 G-A polymorphism influences the mitotic spindle assembly checkpoint, affecting chemotherapy efficacy and survival [17]. SNP rs7804190 is linked to survival outcomes in endometrioid OC [18]. Methylation at CpG site cg16172923 is associated with OS, with higher methylation correlating with better outcomes in relapsed cases [15]. These findings highlight *MAD1L1*'s role in chromosomal abnormalities, chemoresistance, and prognosis in ovarian adenocarcinoma.

Other genes, *ZNF385D*, *SBNO2*, *DUSP6*, *PPP2R5E*, *ZPLD1*, and *SAMD12*, are also relevant for OC prognosis. In relapsed patients, lower methylation at CpG sites correlates with higher gene activity and poorer survival, making these genes valuable for tracking disease progression. Conversely, higher methylation at these sites is associated with improved outcomes, extending survival by an average of 15 months [15]. In another study, *ZNF385D*, as part of a prognostic signature involving m6A-related long non-coding RNAs

(lncRNAs), underscores its role in OC progression and treatment response [16]. *SBNO2* also impacts treatment response and disease progression, showing potential as a prognostic marker comparable to established ones like SRY-box TF 11 (*SOX11*) [41].

In addition to our systematic review, our bioinformatics validation reveals that PLAT-M8 genes—*DUSP6*, *SAMD12*, and *MAD1L1*—are generally overexpressed in OC compared to normal tissue, aligning with James et al [28]. However, in OC cases with poorer prognosis, *DUSP6* and *SAMD12* are underexpressed. Conversely, *PPP2R5E*, *ARID5B*, and *SBNO2* show stable expression levels in both normal and cancerous tissues, but in poorer prognosis cases, *PPP2R5E* and *ARID5B* are overexpressed, while *SBNO2* is underexpressed. *ZNF385D* and *ZPLD1*, underexpressed in OC compared to normal tissues, exhibit increased expression in poorer prognosis cases (**Figure 2** and **3**). Our bioinformatics analysis clarifies previous inconsistencies by using median cutoffs for greater reproducibility, unlike studies with automated or unspecified cutoffs. Moreover, our larger sample size (655–1656 patients for OS, 614–1435 for PFS) offers more robust results compared to smaller studies, such as Gao et al. [30], which linked higher *DUSP6* expression to poor outcomes based on a small sample of 39 OC tissues and 4 cell lines. Our study confirms that lower *DUSP6* expression correlates with worse outcomes, challenging the findings of the smaller study.

Moreover, overexpression of the eight PLAT-M8 genes consistently correlates with poorer outcomes due to their roles in tumorigenesis, progression, metastasis, chemoresistance, and decreased survival. These genes, also effective as blood-based methylation biomarkers, mirror tumour biopsy patterns [15]. Our review highlights their specific roles in OC: *DUSP6* and *PPP2R5E* in tumorigenesis; *ARID5B* in prognosis and progression; *MAD1L1* in prognosis, treatment response, and chemoresistance; *SAMD12* in treatment response and clinicopathological prediction; and *SBNO2*, *ZNF385D*, and *ZPLD1* in prognosis and treatment response. While each gene contributes individually, their combined impact highlights significant pathways, suggesting future research should explore both individual and collective gene effects. Additionally, the role of PLAT-M8 methylation in MMR and its context-dependent effects after platinum exposure remain unclear. Further investigation is needed to clarify these mechanisms and validate PLAT-M8's role in predicting treatment response and prognosis. This research should encompass both epigenomics and transcriptomics, as illustrated in **Figure 6**.

<Figure6>

STRENGTHS AND LIMITATIONS

This study identifies PLAT-M8 as a novel prognostic biomarker for primary OC, enabling early detection of high-risk patients and predicting their prognosis. It reveals key gene expression correlations with PLAT-M8 methylation, exploring their roles in tumorigenesis, chemoresistance, and survival. The study also examines epigenetic mechanisms, demonstrating how PLAT-M8 CpG site methylation affects gene expression. Bioinformatics validation confirms the clinical relevance of PLAT-M8, highlighting its potential to enhance personalised medicine. Limitations include a lack of data on some genes in OC literature and the lack of comprehensive blood datasets, which affects alignment with the biomarker. Despite these limitations, the findings provide valuable insights into the prognostic value of PLAT-M8 gene expression in tumour biopsies at primary diagnosis.

CONCLUSIONS

Current systematic and bioinformatic analysis of TCGA and GSE datasets confirms that the expression of the eight PLAT-M8 genes in tumour biopsies may serve as a prognostic indicator for primary OC. This could provide early prognostic information before methylation changes become apparent in relapse cases following platinum treatment, where PLAT-M8 methylation status is more significant. Among these genes, *DUSP6*, a key PLAT-M8 gene, is crucial for overcoming chemotherapy resistance by modulating the MAPK pathway. Additionally, *PPP2R5E* contributes to tumorigenesis through its PP2A subunit, while *ARID5B* plays a crucial role in prognosis and progression by influencing tumour behaviour through its effects on methylation and gene activity. These findings highlight the potential for more targeted and effective OC treatments. To translate these insights into clinical practice, PLAT-M8 should be validated in larger, diverse patient populations. Further research into the mechanistic roles and methylation profiles of these genes is needed to develop targeted therapies and comprehensive biomarker panels for improved patient stratification and treatment outcomes in OC.

Figure legends

Figure 1. Study design. **(A)** Flowchart depicting the article selection process based on the PRISMA 2020 guidelines for conducting a systematic review and bioinformatics validation using web-based tools and datasets from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA); **(B)** Risk of bias according to Cochrane criteria for interventional study; **(C)** Biomarker toolkit assessment.

Figure 2. Cancer hallmarks for multigene analysis of PLAT-M8 genes based on web-based bioinformatics analysis using TCGA and GSE datasets. **(A)** Violin plot showing gene expression differences between normal and tumour ovary tissues: *ZNF385D* and *ZPLD1* have lower expression in tumours, *MAD1L1* is slightly higher, and *SAMD12* and *DUSP6* are significantly higher in tumours. *ARID5B*, *SBNO2*, and *PPP2R5E* show similar expression levels in both tissue types; **(B)** Targetgram analysis of the 8-gene set shows higher mean values for *SBNO2*, *ARID5B*, *PPP2R5E*, *MAD1L1*, and *DUSP6* in normal tissue, with *SBNO2* showing the highest. In tumour tissue, *SBNO2* again has the highest mean value, followed by *ARID5B*, *DUSP6*, *PPP2R5E*, *MAD1L1*, and *SAMD12*. The size of the segments represents mean values, and the dashed lines indicate median values; **(C)** Gene signature analysis of PLAT-M8-related genes reveals a 1.33-fold change (FC) higher expression in tumour tissue; **(D)** Gene-enriched pathway analysis indicates that PLAT-M8 signature genes are primarily involved in protein dephosphorylation; **(E)** Biological process analysis reveals that PLAT-M8 signature genes are primarily involved in sequence-specific double-stranded DNA binding; **(F)** Cellular component analysis indicates that PLAT-M8 signature genes are mainly involved in the nucleus and membrane-bounded organelles; **(G)** Disease mechanisms analysis identifies replicative immortality and reprogramming energy metabolism as significant drivers of carcinogenesis and progression ($p < 0.001$), with no involvement in sustained angiogenesis, tumour-promoting inflammation, immune evasion, or genome instability. Key contributions include *DUSP6* and *PPP2R5E* in sustained proliferative signalling; *PPP2R5E* in resisting cell death, evading growth suppressors, and reprogramming energy metabolism; *MAD1L1* in replicative immortality; and *DUSP6* in tissue invasion and metastasis. **(H)** The correlation plot for the 8 genes shows minimal overall correlation. Weak correlations are observed between: *SBNO2* and *ZNF385D* & *MAD1L1*; *SAMD12* and *ARID5B* & *DUSP6*; *ARID5B* and *DUSP6* & *PPP2R5E*; *DUSP6* and *PPP2R5E*; and *PPP2R5E* and *SBNO2*.

Figure 3. Forest plot of univariate overall survival (OS) and progression-free survival analysis from mRNA gene chip (microarray) dataset in **(A)** OS analysis in overall cases and **(B)** subgroup analysis based on platinum-only treatment, **(C)** PFS analysis in overall cases and **(D)** subgroup analysis based on platinum-only treatment. The multiple 8-gene signature impacts overall cases in predicting OS and PFS, particularly in platinum-containing treatments, which are prone to relapse. The two genes most predictive of OS are *ZNF385D* and *DUSP6*.

Figure 4. Mechanism of platinum-based chemotherapy inducing methylation and its impact on sensitivity to subsequent chemotherapy. The platinum treatment induces abnormal DNA methylation in ovarian cancer (OC) tissues, detectable in blood samples. Platinum adducts, which form primarily within and between DNA strands, cause significant DNA damage, including single-strand breaks, base mismatches, and crosslinks. This damage disrupts DNA replication and transcription, triggering a mismatch repair (MMR) response. Efficient MMR can lead to apoptosis, increasing chemotherapy sensitivity. However, replication stress may induce aberrant DNA methylation, resulting in either hypermethylation or hypomethylation of genes. Hypermethylation typically silences oncogenes and drug-resistance genes, while in tumour suppressor genes (TSGs), it may paradoxically increase expression. Conversely, hypomethylation in chemo-resistant patients, often due to poor MMR, disrupts normal methylation patterns, leading to increased drug resistance. These changes are influenced by various factors, including gene location and cellular context. **Abbreviations:** *DDR*, DNA Damage Response; *HR*, Homologous Recombination Repair; *MLH*, MutL Homolog; *MSH*, MutS Homolog; *NER*, Nucleotide Excision Repair; *NHEJ*, Non-Homologous End Joining; *Pt*, platinum. The interrogative symbol (?) represents research gaps.

Figure 5. Comprehensive signalling pathways involving PLAT-M8 genes in tumorigenesis, chemoresistance, metastasis, and recurrence: (1) **ZNF385D** interacts with epigenetic regulators (e.g., m6A RNA methylation), promoting EMT and β -catenin signalling, which are linked to metastasis and poor prognosis; (2) **ZNF385D** drives EMT and metastasis and its methylation changes affect survival and treatment response by altering the extracellular matrix and cell adhesion; (3) **MAD1L1** ensures chromosome segregation, and its overexpression causes instability and chemoresistance by enabling apoptosis evasion; (4) **SAMD12** acts as a tumour suppressor, and its reduced expression leads to increased proliferation and poor survival, with methylation changes affecting prognosis and DNA repair; (5) **ARID5B**, a transcription factor that influences chromatin remodelling and gene expression, affects proliferation, migration, and invasion. Variations during treatment indicate its role in adaptive resistance and its promotion of growth and metastasis through the cell cycle and apoptosis regulation; (6) **DUSP6** inhibits ERK signalling, and its loss sustains ERK activation, driving tumour growth and chemoresistance. Lower DUSP6 levels correlate with poorer survival, while its inhibition enhances chemotherapy by promoting apoptosis; (7) **PPP2R5E**, part of the PP2A complex, regulates the cell cycle. Dysregulation causes uncontrolled growth, EMT, and immune evasion, affecting key cancer pathways like Wnt, PI3K/AKT, and MAPK; and (8) **SBNO2** regulates immune responses and inflammation, influencing tumour progression and metastasis by affecting immune cell infiltration and the tumour microenvironment. These genes are crucial in tumorigenesis, progression, metastasis, and chemoresistance, making them

valuable as prognostic markers and therapeutic targets in ovarian cancer. Their complex interactions drive cancer development and impact treatment outcomes. **Abbreviations:** Ac, Acetylated; AKT, Protein Kinase B; AMPK- α , AMP-Activated Protein Kinase Alpha; APC/C, Anaphase-Promoting Complex/Cyclosome; ARID5B, AT-Rich Interaction Domain 5B; ATM, Ataxia Telangiectasia Mutated; ATR, Ataxia Telangiectasia and Rad3-Related; BAD, Bcl-2-Associated Death Promoter; Bcl-2, B-Cell Lymphoma 2; BIM-EL, Bcl-2 Interacting Mediator of Cell Death - Extra Long; BRCA1, Breast Cancer 1; BUB3, Budding Uninhibited by Benzimidazoles 3; BUBR1, Budding Uninhibited by Benzimidazoles 1; CDC20, Cell Division Cycle 20; CHK1/2, Checkpoint Kinase 1/2; c-Myc, Cellular Myelocytomatosis Oncogene; DDR, DNA-Damage Response; DUSP6, Dual Specificity Phosphatase 6; E2F, E2 Transcription Factor; EMT, Epithelial-Mesenchymal Transition; ERK, Extracellular Signal-Regulated Kinase; FGF, Fibroblast Growth Factor; Foxp3, Forkhead Box P3; IL-2R, Interleukin-2 Receptor; IL-6, Interleukin-6; JAK, Janus Kinase; MAD1L1, Mitotic Arrest Deficient 1 Like 1; MAPK, Mitogen-Activated Protein Kinase; MCC, Mitotic Checkpoint Complex; MEK 1, Mitogen-Activated Protein Kinase Kinase 1; MMP, Matrix Metalloproteinase; MSCs, Mesenchymal Stem Cells; mTOR, Mechanistic Target of Rapamycin; Myb, Myb Proto-Oncogene; NANOG, Nanog Homeobox; NF κ B, Nuclear Factor kappa-Light-Chain-Enhancer of Activated B Cells; OS, Overall Survival; P, Phosphorylated; p21, Cyclin-Dependent Kinase Inhibitor 1; P53, Tumour Protein 53; PI3K/AKT, Phosphatidylinositol 3-Kinase/Protein Kinase B; PPP2R5E, Protein Phosphatase 2 Regulatory Subunit B ϵ ; Prx3, Peroxiredoxin 3; Pt, Platinum; RACK1, Receptor for Activated C-Kinase 1; Rb1, Retinoblastoma 1; ROS, Reactive Oxygen Species; SAC, Spindle Assembly Checkpoint (SAC); SAMD, Sterile Alpha Motif Domain Containing; SBNO2, Strawberry Notch Homolog 2; SNAI1, Snail Family Transcriptional Repressor 1; SNAI2, Snail Family Transcriptional Repressor 2; SNAIL, Snail Family Transcriptional Repressor; SOX4, SRY-Box Transcription Factor 4; STAT, Signal Transducer and Activator of Transcription; TF, Transcription Factors; TSG, Tumour Suppressor Genes; VAV3, Vav 3 Oncogene; VEGF, Vascular Endothelial Growth Factor; ZNF385D, Zinc Finger Protein 385D; ZPLD1, Zona Pellucida Like Domain Containing 1; β -catenin, Beta-Catenin.

Figure 6. Proof of concept linking methylation and gene expression for PLAT-M8. Methylation can occur in various genomic regions, including promoters, gene bodies, enhancers, and repeat elements. Typically, promoter hypermethylation is inversely related to gene expression, though both promoter and non-promoter hypermethylation can have context-dependent, sometimes paradoxical effects. PLAT-M8 genes are mainly found in introns and exons, and ongoing research is examining how methylation in these areas affects gene expression, especially comparing blood and tumour tissues. Class 1 clusters, characterised by hypomethylation in blood, are associated with worse outcomes due to the activation of oncogenes, drug-

resistance genes, and global hypomethylation, which causes chromosomal instability and altered cell differentiation. Conversely, hypermethylation may deactivate oncogenes and drug-resistance genes, potentially leading to better outcomes, although effects can be paradoxical. Increased expression of PLAT-M8 genes in Class 1 patients may drive ovarian carcinogenesis, chemoresistance, recurrence, and reduced survival. Future research should explore the impact of PLAT-M8 methylation on non-promoter regions, its role in carcinogenesis, and how methylation affects chemoresistance in both primary and relapsed ovarian cancer, using blood and tumour biopsies. The interrogative symbol (?) denotes existing research gaps.

Supplemental information

Document S1. Figures S1–S4

Document S2. Tables S1–S9

REFERENCES

1. Mazidimoradi, A. *et al.* (2023) Global patterns and temporal trends in ovarian cancer morbidity, mortality, and burden from 1990 to 2019. 25, 641-659
2. Li, S.S. *et al.* (2018) Chemoresistance in ovarian cancer: exploiting cancer stem cell metabolism. *J Gynecol Oncol* 29, e32
3. Song, K. and Artibani, M. (2023) The role of DNA methylation in ovarian cancer chemoresistance: A narrative review. *Health Sci Rep* 6, e1235
4. Matthews, B.G. *et al.* (2021) Epigenetic Mechanisms and Therapeutic Targets in Chemoresistant High-Grade Serous Ovarian Cancer. *Cancers (Basel)* 13,
5. Li, G. *et al.* (2021) The Non-Coding RNAs Inducing Drug Resistance in Ovarian Cancer: A New Perspective for Understanding Drug Resistance. *Front Oncol* 11, 742149
6. Muñoz-Galván, S. and Carnero, A. (2021) Leveraging Genomics, Transcriptomics, and Epigenomics to Understand the Biology and Chemoresistance of Ovarian Cancer. *Cancers (Basel)* 13,
7. Spainhour, J.C. *et al.* (2019) Correlation Patterns Between DNA Methylation and Gene Expression in The Cancer Genome Atlas. *Cancer Inform* 18, 1176935119828776
8. Smith, J. *et al.* (2020) Promoter DNA Hypermethylation and Paradoxical Gene Activation. *Trends Cancer* 6, 392-406
9. Page, M.J. *et al.* (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 372, n71
10. Higgins, J.P.T. *et al.* (2011) The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 343, d5928
11. Savva, K.V. *et al.* (2023) The Biomarker Toolkit - an evidence-based guideline to predict cancer biomarker success and guide development. *BMC Med* 21, 383
12. Bartha, Á. and Györfy, B. (2021) TNMplot.com: A Web Tool for the Comparison of Gene Expression in Normal, Tumor and Metastatic Tissues. *Int J Mol Sci* 22,
13. Menyhart, O. *et al.* (2024) A gene set enrichment analysis for the cancer hallmarks. *Journal of Pharmaceutical Analysis*, 101065
14. Györfy, B. (2023) Discovery and ranking of the most robust prognostic biomarkers in serous ovarian cancer. *Geroscience* 45, 1889-1898
15. Flanagan, J.M. *et al.* (2017) Platinum-Based Chemotherapy Induces Methylation Changes in Blood DNA Associated with Overall Survival in Patients with Ovarian Cancer. *Clinical Cancer Research* 23, 2213-2222

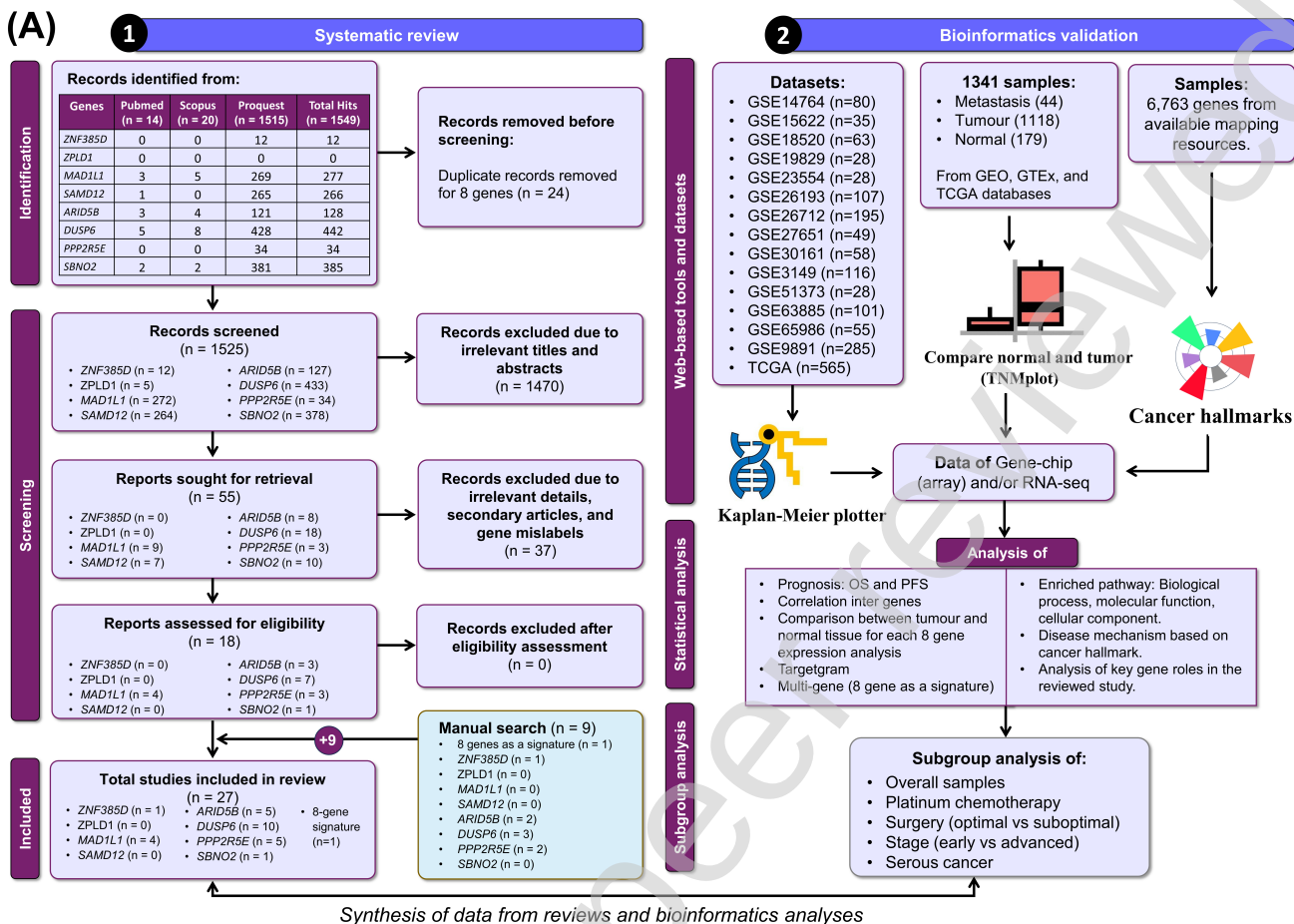
16. Song, Y. and Qu, H. (2022) Identification and validation of a seven m6A-related lncRNAs signature predicting prognosis of ovarian cancer. *BMC Cancer* 22, 633
17. Santibanez, M. *et al.* (2013) The MAD1 1673 G --> A polymorphism alters the function of the mitotic spindle assembly checkpoint and is associated with a worse response to induction chemotherapy and sensitivity to treatment in patients with advanced epithelial ovarian cancer. *Pharmacogenet Genomics* 23, 190-199
18. Goode, E.L. *et al.* (2013) Inherited variants in regulatory T cell genes and outcome of ovarian cancer. *PLoS One* 8, e53903
19. Bandala-Jacques, A. *et al.* (2020) Prognostic Significance of the MAD1L1 1673 G: A Polymorphism in Ovarian Adenocarcinomas. *Rev Invest Clin* 72, 372-379
20. Ju, W. *et al.* (2009) Identification of genes with differential expression in chemoresistant epithelial ovarian cancer using high-density oligonucleotide microarrays. *Oncol Res* 18, 47-56
21. Baba, A. *et al.* (2011) PKA-dependent regulation of the histone lysine demethylase complex PHF2-ARID5B. *Nat Cell Biol* 13, 668-675
22. Chava, S. *et al.* (2021) Disruptor of telomeric silencing 1-like promotes ovarian cancer tumor growth by stimulating pro-tumorigenic metabolic pathways and blocking apoptosis. *Oncogenesis* 10, 48
23. Zhao, H. *et al.* (2021) Single-cell analysis revealed that IL4I1 promoted ovarian cancer progression. *J Transl Med* 19, 454
24. Yan, K. *et al.* (2023) Copy number variants landscape of multiple cancers and clinical applications based on NGS gene panel. *Ann Med* 55, 2280708
25. Deng, Y. *et al.* (2023) ARID5B promoted the histone demethylation of SORBS2 and hampered the metastasis of ovarian cancer. *Pathol Res Pract* 252, 154911
26. Chan, D.W. *et al.* (2008) Loss of MKP3 mediated by oxidative stress enhances tumorigenicity and chemoresistance of ovarian cancer cells. *Carcinogenesis* 29, 1742-1750
27. Cossa, G. *et al.* (2014) Differential outcome of MEK1/2 inhibitor-platinum combinations in platinum-sensitive and -resistant ovarian carcinoma cells. *Cancer Lett* 347, 212-224
28. James, N.E. *et al.* (2019) Inhibition of DUSP6 sensitizes ovarian cancer cells to chemotherapeutic agents via regulation of ERK signaling response genes. *Oncotarget* 10, 3315-3327
29. James, N.E. *et al.* (2019) Human Epididymis Secretory Protein 4 (HE4) Compromises Cytotoxic Mononuclear Cells via Inducing Dual Specificity Phosphatase 6. *Front Pharmacol* 10, 216
30. Gao, Y. *et al.* (2020) Overexpression of DUSP6 enhances chemotherapy-resistance of ovarian epithelial cancer by regulating the ERK signaling pathway. *J Cancer* 11, 3151-3164
31. Gjyshi, A. *et al.* (2018) Early transcriptional response of human ovarian and fallopian tube surface epithelial cells to norepinephrine. *Scientific Reports* 8, 8291
32. Liu, S. *et al.* (2021) Targeting enhancer reprogramming to mitigate MEK inhibitor resistance in preclinical models of advanced ovarian cancer. *J Clin Invest* 131,
33. Chesnokov, M.S. *et al.* (2022) Optimized Transcriptional Signature for Evaluation of MEK/ERK Pathway Baseline Activity and Long-Term Modulations in Ovarian Cancer. *Int J Mol Sci* 23,
34. Sanders, B.E. *et al.* (2022) Targeting DUSP Activity as a Treatment for High-Grade Serous Ovarian Carcinoma. *Mol Cancer Ther* 21, 1285-1295
35. Güler, S. and Yalçın, A. (2023) Expression of dual-specificity phosphatases in TGFβ1-induced EMT in SKOV3 cells. *Turk J Med Sci* 53, 640-646
36. Park, Y.T. *et al.* (2013) MicroRNAs overexpressed in ovarian ALDH1-positive cells are associated with chemoresistance. *J Ovarian Res* 6, 18
37. Wu, C. *et al.* (2018) Identifying miRNA-mRNA regulation network of major depressive disorder in ovarian cancer patients. *Oncol Lett* 16, 5375-5382
38. Penyige, A. *et al.* (2019) Circulating miRNA Profiling in Plasma Samples of Ovarian Cancer Patients. *Int J Mol Sci* 20,
39. Mehdi, S. *et al.* (2020) LY75 Ablation Mediates Mesenchymal-Epithelial Transition (MET) in Epithelial Ovarian Cancer (EOC) Cells Associated with DNA Methylation Alterations and Suppression of the Wnt/β-Catenin Pathway. *Int J Mol Sci* 21,

40. Gandham, S.K. *et al.* (2022) Combination microRNA-based cellular reprogramming with paclitaxel enhances therapeutic efficacy in a relapsed and multidrug-resistant model of epithelial ovarian cancer. *Mol Ther Oncolytics* 25, 57-68
41. Barna, A.J. *et al.* (2023) High Tumor-Infiltrating Lymphocyte Count Is Associated with Distinct Gene Expression Profile and Longer Patient Survival in Advanced Ovarian Cancer. *Int J Mol Sci* 24,
42. Zhao, X. *et al.* (2022) Molecular Mechanisms of ARID5B-Mediated Genetic Susceptibility to Acute Lymphoblastic Leukemia. *J Natl Cancer Inst* 114, 1287-1295
43. Rocha, C.R.R. *et al.* (2018) DNA repair pathways and cisplatin resistance: an intimate relationship. *Clinics (Sao Paulo)* 73, e478s
44. Slysikova, J. *et al.* (2018) Base and nucleotide excision repair facilitate resolution of platinum drugs-induced transcription blockage. *Nucleic Acids Res* 46, 9537-9549
45. Wang, R. *et al.* (2023) Targeting the DNA Damage Response for Cancer Therapy. *International Journal of Molecular Sciences* 24, 15907
46. Huang, R. and Zhou, P.-K. (2021) DNA damage repair: historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. *Signal Transduction and Targeted Therapy* 6, 254
47. Ubhi, T. and Brown, G.W. (2019) Exploiting DNA Replication Stress for Cancer Treatment. *Cancer Res* 79, 1730-1739
48. O'Connor, Mark J. (2015) Targeting the DNA Damage Response in Cancer. *Molecular Cell* 60, 547-560
49. Siegfried, Z. and Simon, I. (2010) DNA methylation and gene expression. *Wiley Interdiscip Rev Syst Biol Med* 2, 362-371
50. Rauluseviciute, I. *et al.* (2020) DNA hypermethylation associated with upregulated gene expression in prostate cancer demonstrates the diversity of epigenetic regulation. *BMC Med Genomics* 13, 6
51. Moarii, M. *et al.* (2015) Changes in correlation between promoter methylation and gene expression in cancer. *BMC Genomics* 16, 873
52. Oliver, V.F. *et al.* (2013) A novel methyl-binding domain protein enrichment method for identifying genome-wide tissue-specific DNA methylation from nanogram DNA samples. *Epigenetics Chromatin* 6, 17
53. Zhu, H. *et al.* (2016) Transcription factors as readers and effectors of DNA methylation. *Nat Rev Genet* 17, 551-565
54. Bahar Halpern, K. *et al.* (2014) Paradoxical role of DNA methylation in activation of FoxA2 gene expression during endoderm development. *J Biol Chem* 289, 23882-23892
55. Irizarry, R.A. *et al.* (2009) The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nat Genet* 41, 178-186
56. Yoder, J.A. *et al.* (1997) Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet* 13, 335-340
57. Rizwana, R. and Hahn, P.J. (1999) CpG methylation reduces genomic instability. *J Cell Sci* 112 (Pt 24), 4513-4519
58. Rhie, S.K. *et al.* (2016) Identification of activated enhancers and linked transcription factors in breast, prostate, and kidney tumors by tracing enhancer networks using epigenetic traits. *Epigenetics Chromatin* 9, 50
59. Pfeifer, G.P. (2018) Defining Driver DNA Methylation Changes in Human Cancer. *Int J Mol Sci* 19,
60. Curradi, M. *et al.* (2002) Molecular mechanisms of gene silencing mediated by DNA methylation. *Mol Cell Biol* 22, 3157-3173
61. Yin, Y. *et al.* (2017) Impact of cytosine methylation on DNA binding specificities of human transcription factors. *Science* 356,
62. Medvedeva, Y.A. *et al.* (2014) Effects of cytosine methylation on transcription factor binding sites. *BMC Genomics* 15, 119
63. Cho, J.W. *et al.* (2022) The importance of enhancer methylation for epigenetic regulation of tumorigenesis in squamous lung cancer. *Exp Mol Med* 54, 12-22
64. Kreibich, E. *et al.* (2023) Single-molecule footprinting identifies context-dependent regulation of enhancers by DNA methylation. *Mol Cell* 83, 787-802.e789

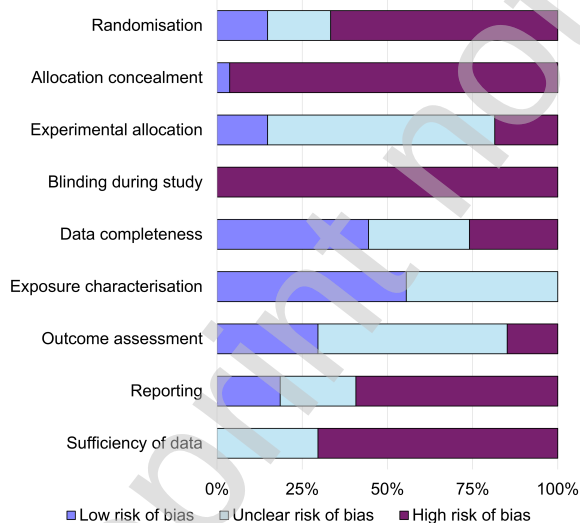
65. Hodges, E. *et al.* (2009) High definition profiling of mammalian DNA methylation by array capture and single molecule bisulfite sequencing. *Genome Res* 19, 1593-1605
66. Eckhardt, F. *et al.* (2006) DNA methylation profiling of human chromosomes 6, 20 and 22. *Nat Genet* 38, 1378-1385
67. Wang, Q. *et al.* (2022) Gene body methylation in cancer: molecular mechanisms and clinical applications. *Clinical Epigenetics* 14, 154
68. Yang, X. *et al.* (2014) Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell* 26, 577-590
69. Nam, A.R. *et al.* (2020) Alternative methylation of intron motifs is associated with cancer-related gene expression in both canine mammary tumor and human breast cancer. *Clinical Epigenetics* 12, 110
70. Lai, A.Y. *et al.* (2010) DNA methylation prevents CTCF-mediated silencing of the oncogene BCL6 in B cell lymphomas. *J Exp Med* 207, 1939-1950
71. Unoki, M. and Nakamura, Y. (2003) Methylation at CpG islands in intron 1 of EGR2 confers enhancer-like activity. *FEBS Lett* 554, 67-72
72. Li, S. *et al.* (2018) Genome-wide analysis reveals that exon methylation facilitates its selective usage in the human transcriptome. *Brief Bioinform* 19, 754-764
73. Wong, C.H. *et al.* (2022) The establishment of CDK9/RNA PolII/H3K4me3/DNA methylation feedback promotes HOTAIR expression by RNA elongation enhancement in cancer. *Mol Ther* 30, 1597-1609
74. Neri, F. *et al.* (2017) Intragenic DNA methylation prevents spurious transcription initiation. *Nature* 543, 72-77
75. Martin, L.P. *et al.* (2008) Platinum resistance: the role of DNA repair pathways. *Clin Cancer Res* 14, 1291-1295
76. Cheung-Ong, K. *et al.* (2013) DNA-damaging agents in cancer chemotherapy: serendipity and chemical biology. *Chem Biol* 20, 648-659
77. Wang, D. and Lippard, S.J. (2005) Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov* 4, 307-320
78. Srivastava, A.K. and Wang, Q.E. (2016) Targeting translesion synthesis to facilitate the eradication of ovarian cancer stem cells by platinum-based therapy. *Mol Cell Oncol* 3, e1043482
79. Haynes, B. *et al.* (2015) Crosstalk between translesion synthesis, Fanconi anemia network, and homologous recombination repair pathways in interstrand DNA crosslink repair and development of chemoresistance. *Mutat Res Rev Mutat Res* 763, 258-266
80. Jin, B. *et al.* (2011) DNA methylation: superior or subordinate in the epigenetic hierarchy? *Genes Cancer* 2, 607-617
81. Hentze, J.L. *et al.* (2019) Methylation and ovarian cancer: Can DNA methylation be of diagnostic use? *Mol Clin Oncol* 10, 323-330
82. Kanwal, A. *et al.* (2016) Hypo/unmethylated promoter status of Cdk2 gene correlates with its over-expression in ovarian cancer in north Indian population. *Cell Mol Biol (Noisy-le-grand)* 62, 67-72
83. Boettcher, M. *et al.* (2010) High-definition DNA methylation profiles from breast and ovarian carcinoma cell lines with differing doxorubicin resistance. *PLoS One* 5, e11002
84. Bram, E.E. *et al.* (2009) Chemotherapeutic drug-induced ABCG2 promoter demethylation as a novel mechanism of acquired multidrug resistance. *Neoplasia* 11, 1359-1370
85. Li, M. *et al.* (2009) Integrated analysis of DNA methylation and gene expression reveals specific signaling pathways associated with platinum resistance in ovarian cancer. *BMC Med Genomics* 2, 34
86. Sung, H.Y. *et al.* (2017) Aberrant Hypomethylation of Solute Carrier Family 6 Member 12 Promoter Induces Metastasis of Ovarian Cancer. *Yonsei Med J* 58, 27-34
87. Zhang, W. *et al.* (2015) DNA hypomethylation-mediated activation of Cancer/Testis Antigen 45 (CT45) genes is associated with disease progression and reduced survival in epithelial ovarian cancer. *Epigenetics* 10, 736-748
88. Sung, H.Y. *et al.* (2014) DNA hypomethylation-mediated overexpression of carbonic anhydrase 9 induces an aggressive phenotype in ovarian cancer cells. *Yonsei Med J* 55, 1656-1663

89. Liao, Y.P. *et al.* (2014) Hypomethylation signature of tumor-initiating cells predicts poor prognosis of ovarian cancer patients. *Hum Mol Genet* 23, 1894-1906
90. Romero-Garcia, S. *et al.* (2020) Role of DNA Methylation in the Resistance to Therapy in Solid Tumors. *Front Oncol* 10, 1152
91. Fang, F. *et al.* (2010) A phase 1 and pharmacodynamic study of decitabine in combination with carboplatin in patients with recurrent, platinum-resistant, epithelial ovarian cancer. *Cancer* 116, 4043-4053
92. Ecker, V. *et al.* (2023) Negative feedback regulation of MAPK signaling is an important driver of chronic lymphocytic leukemia progression. *Cell Reports* 42, 113017
93. Piya, S. *et al.* (2012) DUSP6 is a novel transcriptional target of p53 and regulates p53-mediated apoptosis by modulating expression levels of Bcl-2 family proteins. *FEBS Lett* 586, 4233-4240
94. Wu, C.E. *et al.* (2018) ATM Dependent DUSP6 Modulation of p53 Involved in Synergistic Targeting of MAPK and p53 Pathways with Trametinib and MDM2 Inhibitors in Cutaneous Melanoma. *Cancers (Basel)* 11,
95. Moncho-Amor, V. *et al.* (2019) Role of Dusp6 Phosphatase as a Tumor Suppressor in Non-Small Cell Lung Cancer. *International Journal of Molecular Sciences* 20, 2036
96. Turdo, A. *et al.* (2021) Targeting Phosphatases and Kinases: How to Checkmate Cancer. *Front Cell Dev Biol* 9, 690306
97. Pérez-Sen, R. *et al.* (2019) Dual-Specificity Phosphatase Regulation in Neurons and Glial Cells. *International Journal of Molecular Sciences* 20, E1999
98. Tsukano, K. *et al.* (2022) Xenopus Dusp6 modulates FGF signaling to precisely pattern pre-placodal ectoderm. *Developmental Biology* 488, 81-90
99. Moncho-Amor, V. *et al.* (2019) Role of Dusp6 Phosphatase as a Tumor Suppressor in Non-Small Cell Lung Cancer. *Int J Mol Sci* 20,
100. Momeny, M. *et al.* (2024) DUSP6 inhibition overcomes neuregulin/HER3-driven therapy tolerance in HER2+ breast cancer. *EMBO Molecular Medicine* 16, 1603-1629-1629
101. Gershenson, D.M. *et al.* (2022) Trametinib versus standard of care in patients with recurrent low-grade serous ovarian cancer (GOG 281/LOGS): an international, randomised, open-label, multicentre, phase 2/3 trial. *Lancet* 399, 541-553
102. Khan, M.M. *et al.* (2022) PP2A and Its Inhibitors in Helper T-Cell Differentiation and Autoimmunity. *Frontiers in Immunology* 12, 786857
103. Ciccone, M. *et al.* (2015) From the Biology of PP2A to the PADs for Therapy of Hematologic Malignancies. *Frontiers in Oncology* 5, 21
104. Kashani, E. and Vassella, E. (2022) Pleiotropy of PP2A Phosphatases in Cancer with a Focus on Glioblastoma IDH Wildtype. *Cancers* 14, 5227
105. Perrotti, D. and Neviani, P. (2013) Protein phosphatase 2A: a target for anticancer therapy. *The Lancet. Oncology* 14, e229-238
106. Liang, L.-J. *et al.* (2024) CIP2A induces PKM2 tetramer formation and oxidative phosphorylation in non-small cell lung cancer. *Cell Discovery* 10, 13
107. Arriazu, E. *et al.* (2016) Protein Phosphatase 2A as a Therapeutic Target in Acute Myeloid Leukemia. *Front Oncol* 6, 78
108. Narayan, S. *et al.* (2022) Sensitization of FOLFOX-resistant colorectal cancer cells via the modulation of a novel pathway involving protein phosphatase 2A. *iScience* 25, 104518
109. Tan, T.K. *et al.* (2019) Oncogenic transcriptional program driven by TAL1 in T-cell acute lymphoblastic leukemia. *International Journal of Hematology* 109, 5-17
110. Sanda, T. *et al.* (2012) Core Transcriptional Regulatory Circuit Controlled by the TAL1 Complex in Human T Cell Acute Lymphoblastic Leukemia. *Cancer Cell* 22, 209-221
111. Sarkar, S. *et al.* (2021) Mitotic checkpoint defects: en route to cancer and drug resistance. *Chromosome Research* 29, 131-144
112. Cassandri, M. *et al.* (2017) Zinc-finger proteins in health and disease. *Cell Death Discovery* 3, 17071
113. Jen, J. and Wang, Y.-C. (2016) Zinc finger proteins in cancer progression. *Journal of Biomedical Science* 23, 53
114. Iyer, A.S. *et al.* (2023) The Roles of Zinc Finger Proteins in Colorectal Cancer. *International Journal of Molecular Sciences* 24, 10249

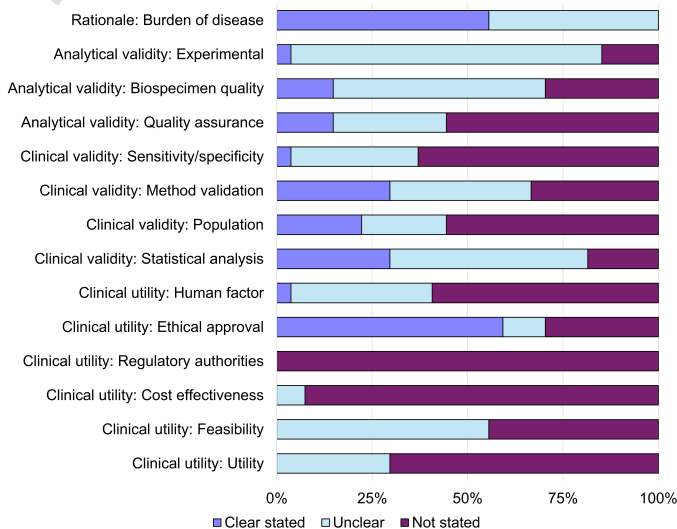
115. Wang, Y. *et al.* (2024) ZNF217: An Oncogenic Transcription Factor and Potential Therapeutic Target for Multiple Human Cancers. *Cancer Manag Res* 16, 49-62
116. Brandstoetter, T. *et al.* (2023) SBNO2 is a critical mediator of STAT3-driven hematological malignancies. *Blood* 141, 1831-1845
117. Syme, T.E. *et al.* (2022) Strawberry notch homolog 2 regulates the response to interleukin-6 in the central nervous system. *Journal of Neuroinflammation* 19, 126
118. Chen, Y.-C. *et al.* (2019) Mesenchymal Stem/Stromal Cell Engulfment Reveals Metastatic Advantage in Breast Cancer. *Cell Reports* 27, 3916-3926.e3915

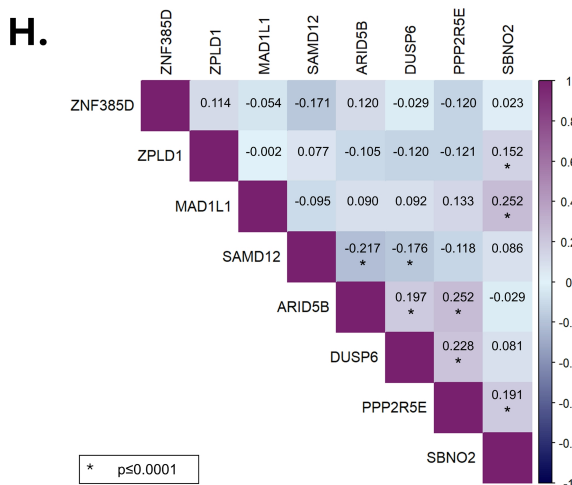
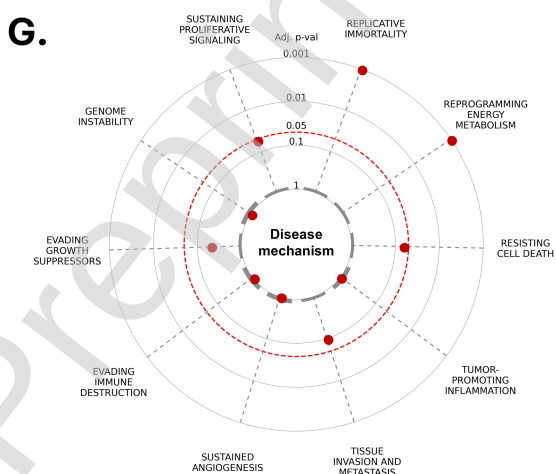
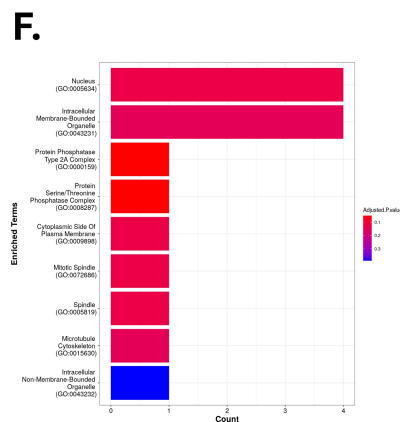
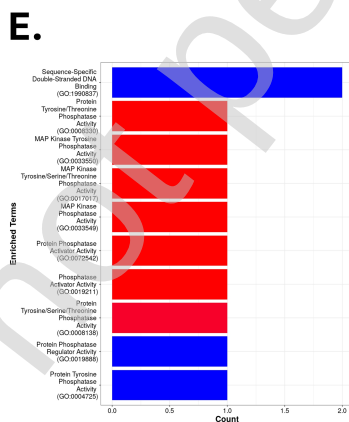
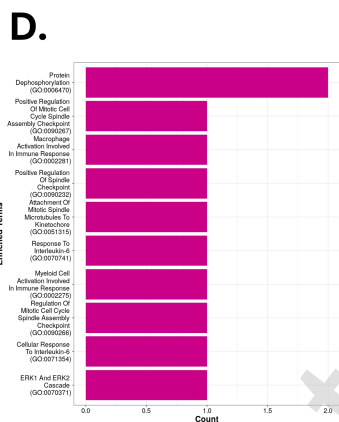
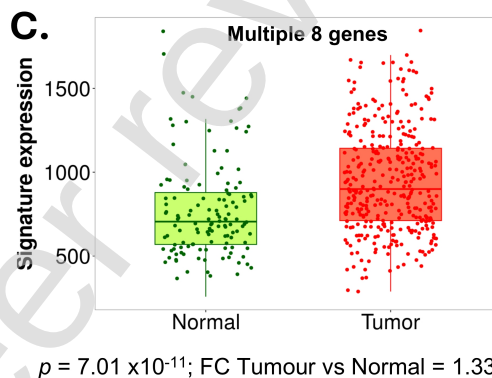
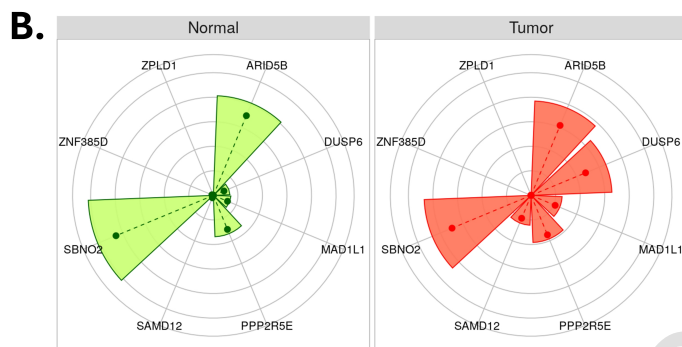
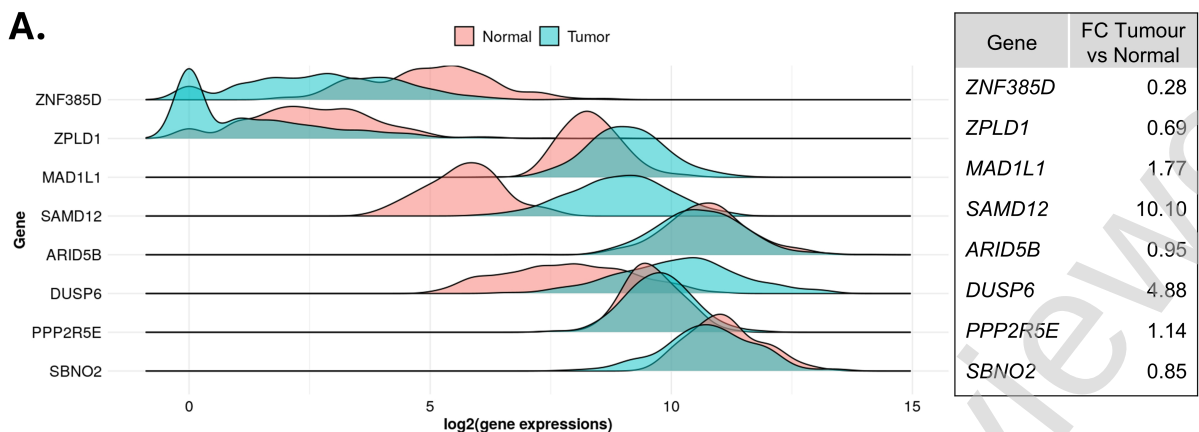


(B) Cochrane Risk of Bias Assessment



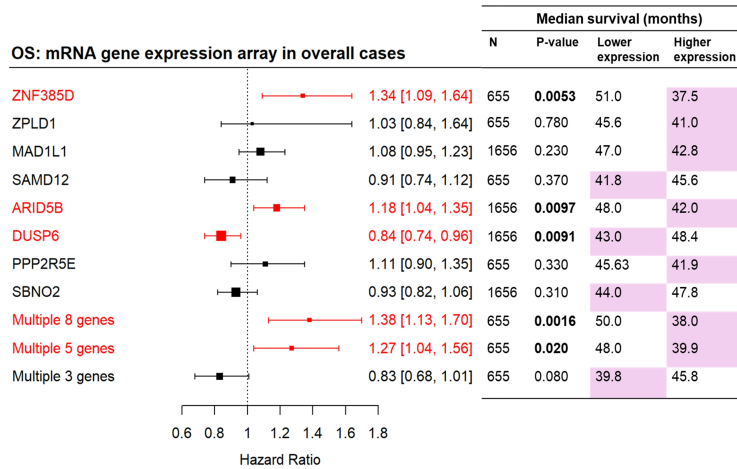
(C) Biomarker Toolkit Assessment





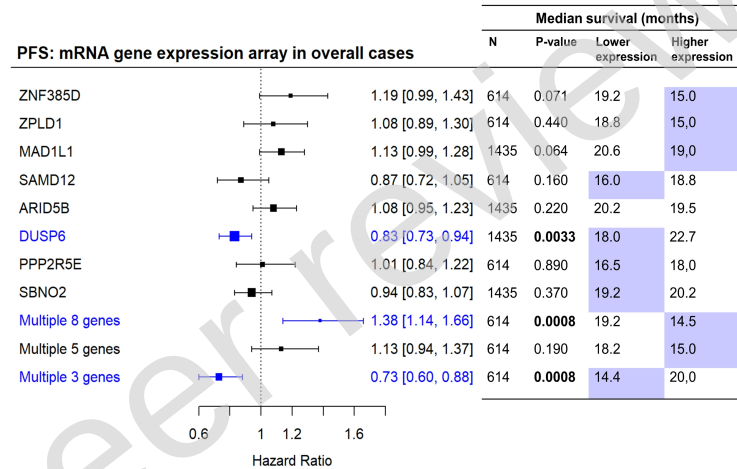
Overall survival

A. All cases

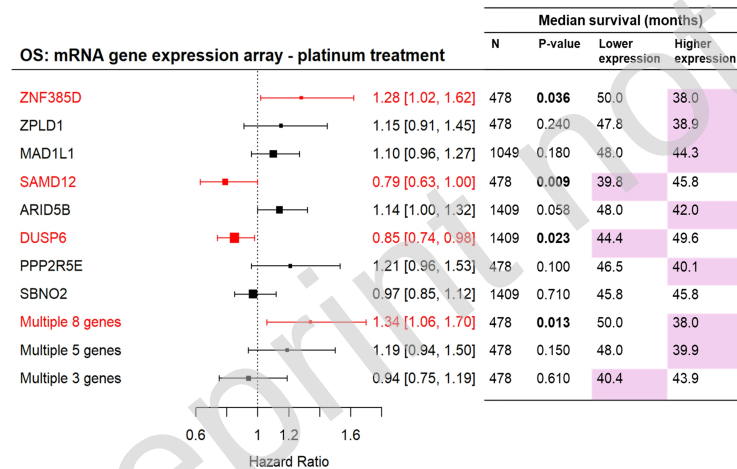


Progression-free survival

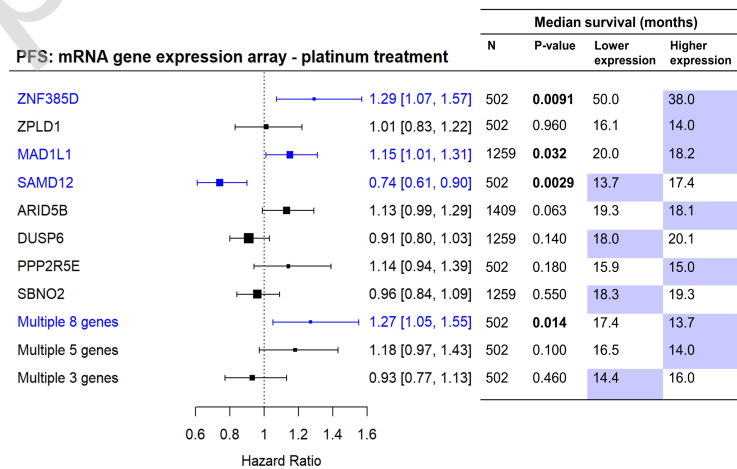
C. All cases



B. Cases receiving treatment containing platinum

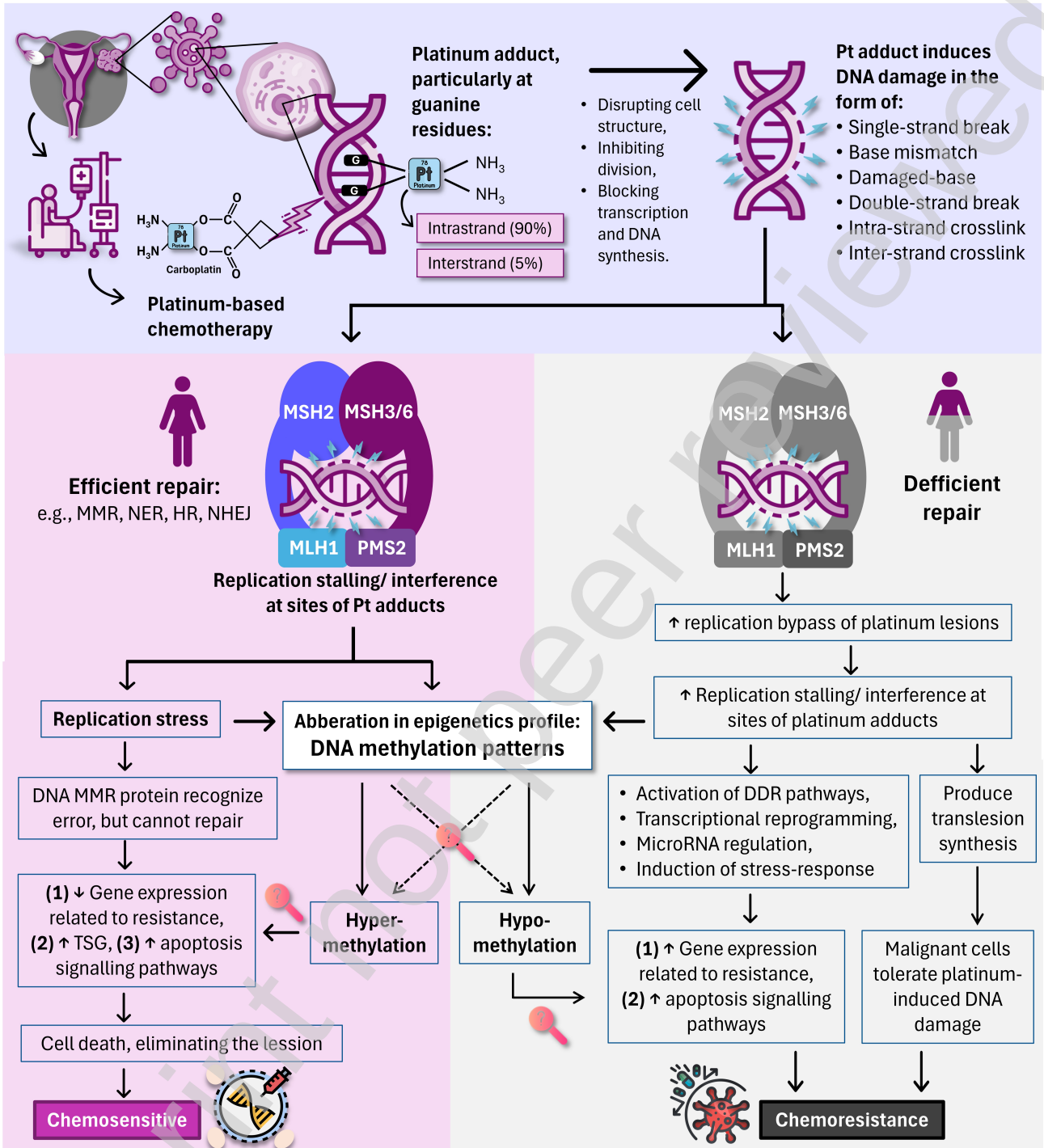


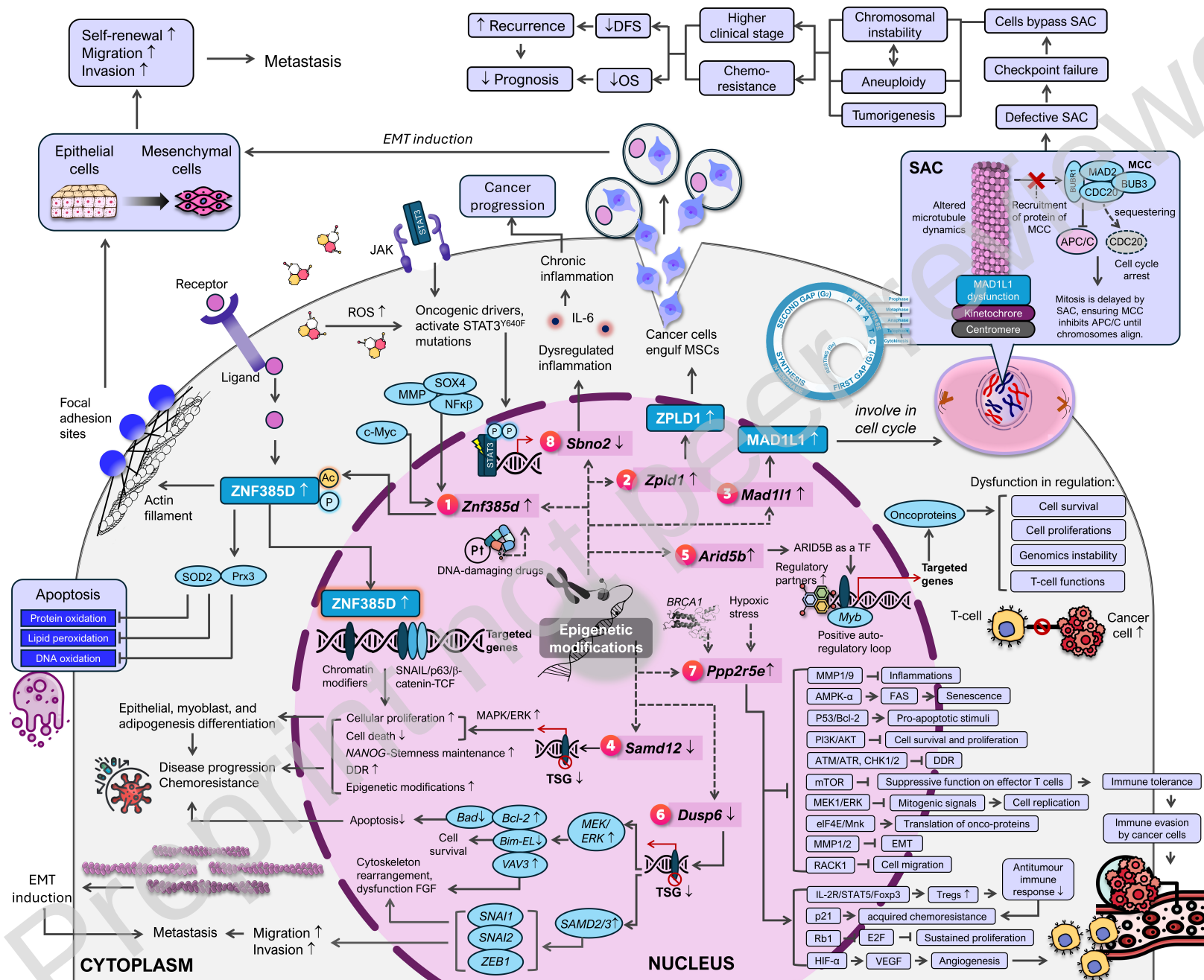
D. Cases receiving treatment containing platinum



Multiple 8 genes: ZNF385D, ZPLD1, MAD1L1, ARID5B, PPP2R5E, SAMD12, DUSP6, SBN02
 Multiple 5 genes: ZNF385D, ZPLD1, MAD1L1, ARID5B, PPP2R5E
 Multiple 3 genes: SAMD12, DUSP6, SBN02

Red colour in the table indicates a poorer prognosis
 NA results signify insufficient data





Proof-of-concept

Gene (DNA)



The methylation impact on the expression of 8 specific genes in ovarian tissue, specific to these regions, is still unknown.

Hypomethylation

Gene activation

Oncogenes (activated)

Genome-wide methylation

Oncogenes (deactivated)

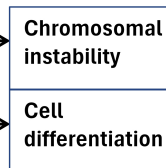
Tumour suppressor genes deactivated or activated [a paradox effect]

Gene silencing

Hypermethylation

Hypothesis:
Influences the expression of PLAT-M8-related genes

Potentially resulting in more unfavorable outcomes



In tumorigenesis, ↑

In tumorigenesis, ↓

Potentially resulting in more favorable outcomes

Depends on:
tissue type, gene location, site functionality, regulatory roles, and interactions with transcription factors

Proposed mechanisms of PLAT-M8

1. Ovary carcinogenesis

- Denovo from normal epithelial cells in the fallopian tube
- Reactivated dormant cells

2. Chemoresistance development

- DNA repair mechanisms, drug efflux pumps, cancer stem cells, and epithelial-to-mesenchymal transition (EMT) ↑
- Apoptosis ↓

Pt resistance

Recurrence

Decrease survival

Research gaps

Condition: Hypomethylated PLAT-M8 signatures (Class 1)

Gene expressions:

↑ PI3K/Akt or MAPK/ERK pathway → cell proliferation.

↑ cell cycle progression and apoptosis evasion.

↑ genomic instability and aneuploidy.

↓ apoptosis, ↑ cell survival.

↑ transcriptional programs → cell cycle control and DNA repair.

↑ MAPK/ERK signaling → cell proliferation.

↑ cell cycle dysregulation, ↑ DNA repair.

↑ immune response dysregulation → tumor progression.

↑ ZNF385D
cg05529343

↑ ZPLD1
cg12992827

↑ MAD1L1
cg16172923

↓ SAMD12
cg07960624

↑ ARID5B
cg25953130

↓ DUSP6
cg13691961

↑ PPP2R5E
cg01692018

↓ SBNO2
cg07573872

Aberant DNA methylation



Increased expression – poorer prognosis

Decreased expression – poorer prognosis

I Intron E Exon ? Hypothesis

Table 1. Characteristics of studies, including authors, publication years, countries, sample sizes, genes studied, molecules investigated, gene roles, and study assessment results.

No	Author, Year, Ref	First author country	Study design	Sample	Sample size	Genes	Investigated molecules	Roles of genes							Assessment	
								TG	RF	DP	PF	LM	CC	TR	RoB ^a	Quality ^b
1.	Flanagan et al., 2017, [1]	UK	Cohort + experimental <i>in vitro</i> + bioinformatics + FO (transcriptomics, genomics, epigenomics)	DNA derived from peripheral blood, tumour samples, and cancer cell lines.	Peripheral blood DNA (n=247) and tumour DNA samples (n=46), and OC cell lines (A2780, A2780/CP70, CP70/MLH1+, CP70/MLH1).	<i>ZNF385D</i> , <i>ZPLD1</i> , <i>MAD1L1</i> , <i>SAMD12</i> , <i>ARID5B</i> , <i>DUSP6</i> , <i>PPP2R5E</i> , <i>SBNQ2</i>	Gene (DNA methylation)				✓		✓	✓	moderate	high
2.	Song et al., 2022, [2]	China	Bioinformatics + FO (transcriptomics)	Public databases and clinical specimens.	TCGA dataset (n=379); Validation cohorts: GSE9891 (n=285), GSE26193 (n=107), and 60 clinical specimens.	<i>ZNF385D</i>	Gene (lncRNA)	✓			✓				moderate	moderate
3.	Santibáñez et al., 2013, [3]	Mexico	Cohort + FO (genomics)	Blood-derived and tumour-derived DNA	Blood-derived DNA: healthy individuals (n=144) and advanced EOC patients (n=91); Samples for treatment response: blood (n=74) and FFPE (n=27).	<i>MAD1L1</i>	Gene (polymorphism) and protein				✓		✓	✓	moderate	high
4.	Goode et al., 2013, [4]	US	Cohort + FO (genomics)	Tumour-derived DNA	DNA samples from Invasive primary EOC cases (n=1,000) and 2,600 cases from FOCI collaboration (n=2,600).	<i>MAD1L1</i>	Gene (mRNA)	✓			✓				high	low
5.	Bandala-Jacques et al., 2019, [5]	Mexico	Cohort + FO (genomics)	Blood-derived DNA	118 patients with ovarian tumours.	<i>MAD1L1</i>	Gene (polymorphism) and protein	✓	✓		✓		✓	✓	moderate	moderate
6.	Ju et al., 2019, [6]	South Korea	Cross-sectional + FO (transcriptomics)	Tumour-derived DNA	13 primary epithelial OC tissues: 5 chemosensitive tumours and 8 chemoresistant tumours	<i>MAD1L1</i>	Gene (mRNA)	✓						✓	high	moderate
7.	Baba et al., 2011, [7]	Japan	Experimental <i>in vivo</i> + <i>in vitro</i> and + FO (genomics and epigenomics)	Cancer cell lines	Cell lines (293F, Sf9) and hepatocytes; cell lysates from 293F cells transfected with plasmids; and chromatin fractions for <i>in vivo</i> histone demethylation assays	<i>ARID5B</i>	Gene (mRNA)	✓		✓	✓	✓			high	low
8.	Chava et al., 2021, [8]	US	Experimental <i>in vivo</i> + <i>in vitro</i> and + bioinformatics + FO (transcriptomics)	Cancer cell lines	12 samples of IGROV-1 and SK-OV-3 cell lines, each with control and treated groups, and 3 replicates per group.	<i>ARID5B</i>	Gene (mRNA)	✓		✓	✓				high	low
9.	Zhao et al., 2021 [9]	China	Bioinformatics + FO (transcriptomics)	Single-cell RNA sequencing datasets	OC single-cell RNA sequencing datasets: TCGA (n=308), GSE (n=906), Oncomine (n=372).	<i>ARID5B</i>	Gene (mRNA)	✓		✓	✓	✓			high	moderate
10.	Yan et al., 2023, [10]	China	Bioinformatics + FO (transcriptomics and genomics)	Blood-derived DNA	1438 Chinese patients across 15 cancer types, including OC (n=28).	<i>ARID5B</i>	Gene (CNV)	✓			✓			✓	high	low
11.	Deng et al., 2023, [11]	China	Cohort + experimental <i>in vivo</i> and <i>in vitro</i> + bioinformatics + FO (genomics and proteomics)	Tumour-derived DNA	33 tissue samples from patients diagnosed with primary OC	<i>ARID5B</i>	Gene (histone modification, mRNA) and protein	✓				✓	✓		moderate	moderate
12.	Chan et al., 2008, [12]	Hongkong	Experimental <i>in vivo</i> and <i>in vitro</i> + FO (transcriptomics)	DNA derived from tumour samples and cancer cell lines.	Primary OC tissues (n=77); normal ovary samples (n=41); cell lines (OV2008, C13, A2780s, A2780cp, DOV13-5, OVCAR3, SKOV3, OV420, OV429, and OV433).	<i>DUSP6</i>	Gene (mRNA)	✓		✓				✓	moderate	low
13.	Cossa et al., 2014, [13]	Italy	Experimental <i>in vitro</i> + FO (transcriptomics)	Cancer cell lines	OC cell lines (IGROV-1, IGROV-1/Pt1, IGROV-1/OHP, and IGROV-1/CP).	<i>DUSP6</i>	Gene (mRNA)	✓						✓	high	low

14.	James et al. (a), 2019, [14]	US	Experimental <i>in vitro</i> + FO (transcriptomics)	Cancer cell lines	OC cell lines: SKOV3 and OVCAR8	DUSP6	Gene (mRNA)	✓						✓	moderate	low
15.	James et al. (b), 2019, [15]	US	Experimental <i>in vitro</i> + FO (transcriptomics)	PBMCs and cancer cell lines	Primary human PBMCs from four volunteers; ovarian cell lines (SKOV3), NK cell lines (NK-92MI), and T cell lines (TALL-104, and H9).	DUSP6	Gene (mRNA)	✓		✓				✓	moderate	low
16.	Gao et al., 2020, [16]	China	Experimental <i>in vitro</i> + bioinformatics + FO (transcriptomics)	DNA derived from tumour samples and cancer cell lines.	Primary OC tissues (n=39) and OC cell lines (HO8910, OVCAR8, SKOV3, OVCAR3).	DUSP6	Gene (mRNA)	✓		✓				✓	moderate	moderate
17.	Gjyshi et al., 2018 [17]	US	Experimental <i>in vitro</i> + bioinformatics FO (transcriptomics and proteomics)	Cancer cell lines	Immortalised epithelial cells: iOSE11 (ovarian), iFTSEC283 (fallopian tube).	DUSP6	Gene (mRNA) and protein	✓		✓				✓	moderate	low
18.	Liu et al., 2021, [18]	China	Experimental <i>in vivo</i> and <i>in vitro</i> + FO (transcriptomics and genomics)	DNA derived from tumour samples and primary cell cultures	Patient tumour tissues for PDX models and primary cell cultures; and OC cell lines (A2780, COV362, COV504, KURAMOCHI, and DOV13).	DUSP6	Gene (mRNA)							✓	moderate	moderate
19.	Chesnokov et al., 2022, [19]	US	Experimental <i>in vitro</i> + bioinformatics + FO (transcriptomics)	Cancer cell lines	OC cell lines: OVCAR8, PEO4, A2780, OVCAR3, OVCAR5, OVCAR7, OVCAR10, OVSAHO, ES2, Pt152, and Pt486.	DUSP6	Gene (mRNA)	✓						✓	moderate	low
20.	Sanders et al., 2022, [20]	US	Experimental <i>in vivo</i> and <i>in vitro</i> + bioinformatics + FO (transcriptomics and proteomics)	PDX, ascites-associated tumour cells, tumour tissues, cancer cell lines, and public dataset.	HGSOC PDX models treated with DUSPi for antitumoural effects, PDX-GTFB1016 ascites cells cultured, HGSOC tumour samples, cancer cell lines (tFTE cells, olaparib-sensitive and olaparib-resistant HGSOC cell lines), and TCGA datasets.	DUSP6	Gene (mRNA) and protein	✓			✓			✓	moderate	moderate
21.	Güler et al., 2023, [21]	Türkiye	Experimental <i>in vitro</i> + bioinformatics + FO (transcriptomics and proteomics)	Cancer cell lines	OC cell line (SKOV3).	DUSP6	Gene (mRNA) and protein	✓		✓				✓	high	low
22.	Park et al., 2013, [22]	South Korea	Cohort + experimental <i>in vitro</i> + FO (transcriptomics)	DNA derived from tumour samples and cancer cell lines.	OC cell line (SKOV3); PTX-resistant cell lines (SKpac-8, -10, -11, -12, -13, -16, -17); primary tumour cells from ovarian serous carcinoma patients; and clinical data of ovarian carcinomas patients (n=34).	PPP2R5E	Gene (mRNA)	✓						✓	high	low
23.	Wu et al., 2018, [23]	China	Bioinformatics + FO (transcriptomics)	Public databases on gene expressions	OC samples (GSE61741): 94 normals, 24 cancer. MDD samples (GSE58105): 11 normals, 14 MDD. Depressed patients with ovarian tumours (SE9116): 5 depressed, 5 non-depressed, all with ovarian tumours.	PPP2R5E	Gene (miRNA and mRNA)	✓	✓	✓					high	low
24.	Penyige et al., 2019, [24]	Hungary	Cross-sectional + bioinformatics + FO (transcriptomics)	Plasma-derived DNA from patients and healthy females.	Plasma miRNAs from 6 healthy controls and 18 serous OC patients.	PPP2R5E	Gene (miRNA)	✓		✓	✓				high	moderate
25.	Mehdi et al., 2020, [25]	Canada	Experimental <i>in vitro</i> + FO (transcriptomics and epigenomics)	Cancer cell lines	OC cell lines: SKOV3-M and SKOV3-E (mesenchymal and epithelial clones); OVCAR8-M (control) and OVCAR8-E (sh-63) clones.	PPP2R5E	Gene (miRNA)	✓		✓		✓			high	low
26.	Gandham et al., 2022, [26]	US	Experimental <i>in vivo</i> and <i>in vitro</i> + FO (transcriptomics)	Cancer cell lines	ID8-VEGF OC cells (model for relapsed EOC and post-chemotherapy multidrug resistance).	PPP2R5E	Gene (miRNA)							✓	high	low
27.	Barna et al., 2023, [27]	Hungary	Cohort + bioinformatics + FO (transcriptomics)	RNA samples derived from FFPE tissues.	RNA samples from 22 OC tumours.	SBN02	Gene (mRNA)	✓			✓				high	moderate

Abbreviations: CC, Clinicopathological characteristic association; DP, Disease progression; EOC, Epithelial ovarian carcinoma; FFPE, Formalin-fixed paraffin-embedded; FO, Functional omics; FOIC, Follow-up of Ovarian Cancer Genetic Association and Interaction Studies; GSE, Gene Set Enrichment; LM, Linked to

metastasis; LncRNA, Long non-coding RNA; PDX, Patient-derived xenograft models; OC, Ovarian cancer; PF, Prognostic factor; RF, Risk factor; TCGA, The Cancer Genome Atlas Program; TG, Tumourigenesis and carcinogenesis; TR, Treatment response including drug resistance.

^aCochrane Risk of Bias (RoB) assessment: 1-6 (high RoB), 7-12 (moderate RoB), 13-18 (low RoB);

^bStudy quality based on the perspective of the Biomarker Toolkit Assessment Tool: 1-9 (low), 10-18 (moderate), 19-28 (high).

References

1. Flanagan, J.M. *et al.* (2017) Platinum-Based Chemotherapy Induces Methylation Changes in Blood DNA Associated with Overall Survival in Patients with Ovarian Cancer. *Clinical Cancer Research* 23, 2213-2222
2. Song, Y. and Qu, H. (2022) Identification and validation of a seven m6A-related lncRNAs signature predicting prognosis of ovarian cancer. *BMC Cancer* 22, 633
3. Santibanez, M. *et al.* (2013) The MAD1 1673 G --> A polymorphism alters the function of the mitotic spindle assembly checkpoint and is associated with a worse response to induction chemotherapy and sensitivity to treatment in patients with advanced epithelial ovarian cancer. *Pharmacogenet Genomics* 23, 190-199
4. Goode, E.L. *et al.* (2013) Inherited variants in regulatory T cell genes and outcome of ovarian cancer. *PLoS One* 8, e53903
5. Bandala-Jacques, A. *et al.* (2020) Prognostic Significance of the MAD1L1 1673 G: A Polymorphism in Ovarian Adenocarcinomas. *Rev Invest Clin* 72, 372-379
6. Ju, W. *et al.* (2009) Identification of genes with differential expression in chemoresistant epithelial ovarian cancer using high-density oligonucleotide microarrays. *Oncol Res* 18, 47-56
7. Baba, A. *et al.* (2011) PKA-dependent regulation of the histone lysine demethylase complex PHF2-ARID5B. *Nat Cell Biol* 13, 668-675
8. Chava, S. *et al.* (2021) Disruptor of telomeric silencing 1-like promotes ovarian cancer tumour growth by stimulating pro-tumorigenic metabolic pathways and blocking apoptosis. *Oncogenesis* 10, 48
9. Zhao, H. *et al.* (2021) Single-cell analysis revealed that IL411 promoted ovarian cancer progression. *J Transl Med* 19, 454
10. Yan, K. *et al.* (2023) Copy number variants landscape of multiple cancers and clinical applications based on NGS gene panel. *Ann Med* 55, 2280708
11. Deng, Y. *et al.* (2023) ARID5B promoted the histone demethylation of SORBS2 and hampered the metastasis of ovarian cancer. *Pathol Res Pract* 252, 154911
12. Chan, D.W. *et al.* (2008) Loss of MKP3 mediated by oxidative stress enhances tumorigenicity and chemoresistance of ovarian cancer cells. *Carcinogenesis* 29, 1742-1750
13. Cossa, G. *et al.* (2014) Differential outcome of MEK1/2 inhibitor-platinum combinations in platinum-sensitive and -resistant ovarian carcinoma cells. *Cancer Lett* 347, 212-224
14. James, N.E. *et al.* (2019) Inhibition of DUSP6 sensitizes ovarian cancer cells to chemotherapeutic agents via regulation of ERK signalling response genes. *Oncotarget* 10, 3315-3327
15. James, N.E. *et al.* (2019) Human Epididymis Secretory Protein 4 (HE4) Compromises Cytotoxic Mononuclear Cells via Inducing Dual Specificity Phosphatase 6. *Front Pharmacol* 10, 216

16. Gao, Y. *et al.* (2020) Overexpression of DUSP6 enhances chemotherapy-resistance of ovarian epithelial cancer by regulating the ERK signalling pathway. *J Cancer* 11, 3151-3164
17. Gijshi, A. *et al.* (2018) Early transcriptional response of human ovarian and fallopian tube surface epithelial cells to norepinephrine. *Scientific Reports* 8, 8291
18. Liu, S. *et al.* (2021) Targeting enhancer reprogramming to mitigate MEK inhibitor resistance in preclinical models of advanced ovarian cancer. *J Clin Invest* 131,
19. Chesnokov, M.S. *et al.* (2022) Optimized Transcriptional Signature for Evaluation of MEK/ERK Pathway Baseline Activity and Long-Term Modulations in Ovarian Cancer. *Int J Mol Sci* 23,
20. Sanders, B.E. *et al.* (2022) Targeting DUSP Activity as a Treatment for High-Grade Serous Ovarian Carcinoma. *Mol Cancer Ther* 21, 1285-1295
21. Güler, S. and Yalçın, A. (2023) Expression of dual-specificity phosphatases in TGFβ1-induced EMT in SKOV3 cells. *Turk J Med Sci* 53, 640-646
22. Park, Y.T. *et al.* (2013) MicroRNAs overexpressed in ovarian ALDH1-positive cells are associated with chemoresistance. *J Ovarian Res* 6, 18
23. Wu, C. *et al.* (2018) Identifying miRNA-mRNA regulation network of major depressive disorder in ovarian cancer patients. *Oncol Lett* 16, 5375-5382
24. Penyige, A. *et al.* (2019) Circulating miRNA Profiling in Plasma Samples of Ovarian Cancer Patients. *Int J Mol Sci* 20,
25. Mehdi, S. *et al.* (2020) LY75 Ablation Mediates Mesenchymal-Epithelial Transition (MET) in Epithelial Ovarian Cancer (EOC) Cells Associated with DNA Methylation Alterations and Suppression of the Wnt/β-Catenin Pathway. *Int J Mol Sci* 21,
26. Gandham, S.K. *et al.* (2022) Combination microRNA-based cellular reprogramming with paclitaxel enhances therapeutic efficacy in a relapsed and multidrug-resistant model of epithelial ovarian cancer. *Mol Ther Oncolytics* 25, 57-68
27. Barna, A.J. *et al.* (2023) High Tumor-Infiltrating Lymphocyte Count Is Associated with Distinct Gene Expression Profile and Longer Patient Survival in Advanced Ovarian Cancer. *Int J Mol Sci* 24,

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw and analysed data of ovarian cancer embedded in the website for tumor vs. normal gene expression analysis.	Bartha and Györfy [1] and TNMplot tool website	GEO, GTEx, and TCGA databases in: https://tnmplot.com/analysis/
Raw and analysed data of ovarian cancer embedded in the website for cancer hallmark analysis.	Menyhart et al. [2] and Cancer hallmarks website	Integrated cancer hallmark gene set in: https://cancerhallmarks.com/
Raw and analysed data of ovarian cancer embedded in the website for survival analysis.	Györfy et al. [3] and KMplotter website	GEO: GSE14764, GSE15622, GSE18520, GSE19829, GSE23554, GSE26193, GSE26712, GSE27651, GSE30161, GSE3149, GSE51373, GSE63885, GSE65986, GSE9891, and TCGA in: https://kmplot.com/
Literature database resources	Pubmed, Scopus, Proquest, and Google Scholar	https://pubmed.ncbi.nlm.nih.gov/ , https://www.scopus.com/sources , https://www.proquest.com/ , https://scholar.google.co.uk/ , and Table S1
Software and algorithms		
Preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines	Page et al. [4]	https://doi.org/10.1136/bmj.n71
Cochrane risk of bias (RoB) assessment tool	Higgins et al. [5]	Table S3 and https://doi.org/10.1136/bmj.d5928
Biomarker Toolkit Assessment	Savva et al. [6]	Table S4 and https://doi.org/10.1186/s12916-023-03075-3
R v4.4.0	This paper and website	https://cran.r-project.org/bin/windows/base/old/4.0.0/
RStudio v2024.04.2	This paper and website	https://posit.co/download/rstudio-server/
EndNote v21.3	University software license	https://imperial.ac.uk/admin-services/library/learning-support/reference-management/endnote/
Microsoft 365 v2406	University software license	https://www.imperial.ac.uk/admin-services/ict/self-service/connect-communicate/office-365/
UCSC Genome Browser	This paper	https://genome.ucsc.edu/
EWAS Atlas@ EWAS Open Platform	This paper	https://ngdc.cncb.ac.cn/ewas/atlas/index

RESOURCE AVAILABILITY

Lead contact

For additional information or requests for resources and reagents, please contact the Lead Investigator, Dr James Flanagan (j.flanagan@imperial.ac.uk), who will handle such inquiries.

Materials availability

This study did not generate any new unique reagents.

Data and code availability

The literature retrieved from PubMed, Scopus, and ProQuest was open source. Datasets for bioinformatics analysis of normal vs. tumor, cancer hallmarks, and survival analysis of the genes of interest are publicly available and can be browsed on the respective bioinformatics websites. They are also easily reproducible. Additional outputs from the analysis are provided in **Figures S1-S4**. Forest plot visualizations for OS and PFS were created in R v4.4.0 and RStudio v2024.04.2 using the *meta*, *metafor*, *ggplot2*, and *grid* packages. Correlation plots were generated with the *corrplot* package, and other visualizations were obtained directly from web-based tools. This study did not generate any new datasets and code. Any additional information required to reanalyse the data reported in this paper are available from the lead contact upon request.

METHOD DETAILS

Protocol, information sources, and search strategy

This systematic review, using a descriptive qualitative approach, adhered to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [4]. The search aimed to explore the role of PLAT-M8-relevant genes in carcinogenesis, chemoresistance, and relapse in OC. The names of the genes were derived from CpG sites listed in Flanagan et al [7], which referenced the UCSC Genome Browser and EWAS Atlas for precise gene names and their locations within the gene body. After listing the genes, literature searches were conducted starting on July 12, 2024, using PubMed, ProQuest, and Scopus. Simple keywords with only AND/OR operators were used to ensure inclusivity and accommodate the novelty of the

topic. PubMed search utilised "MeSH Terms" combined with Boolean operators for specific genes and their aliases ('*ZNF385D*'; '*ZPLD1*'; '*MAD1L1*'; '*SAMD12*'; '*ARID5B*'; '*DUSP6*'; '*PPP2R5E*'; or '*SBNO2*'). Simple search queries for Proquest and Scopus included terms like "ovary," "cancer," and the names of genes and their aliases. A manual search through Google Scholar was also performed to gather additional papers (**Table S1** and **S2**).

Study selection and data collection

The studies were selected based on eligibility criteria using the PICO strategy: Population (OC), Importance/Intervention (genes: *ZNF385D*, *ZPLD1*, *MAD1L1*, *SAMD12*, *ARID5B*, *DUSP6*, *PPP2R5E*, *SBNO2*), Comparator (control group), and Outcome (carcinogenesis, chemoresistance, clinicopathological characteristics, prognostic factors, treatment outcomes including relapse). Inclusion criteria: i) English-language articles; ii) clinical studies, including observational studies assessing the genes of interest; iii) outcomes related to gene expression's impact on progression and prognosis. Exclusion criteria: i) Duplicated articles; ii) abstracts and reviews lacking specific data; iii) articles not meeting study design; iv) preclinical studies, systematic reviews, case reports, theses, letters, editorials, opinions, surveys, guidelines, conference abstracts, and commentaries; v) published within the last sixteen years (2008–2023). The authors independently reviewed titles and abstracts, eliminating duplicates. Any conflicts were resolved by discussions. Eligible articles had their full texts examined. Non-English studies, those not aligning with clinical questions, outdated publications, and duplicates were excluded. Studies unavailable in full text were excluded from the review but used to support discussions.

Quality assessment and risk of bias

We assessed all included studies for risk of bias (RoB) using Cochrane Review tools for interventional studies, covering nine parameters: randomisation, allocation concealment, experimental conditions, blinding, data completeness, exposure characterisation, outcome assessment, selective reporting, and data sufficiency (**Table S3**) [5]. Responses were categorised as low risk (yes), unclear risk, or high risk (no). Each study received a score (yes: 2, unclear: 1, no: 0), with scores ranging from 1–6 (high RoB), 7–12 (moderate

RoB), to 13–18 (low RoB). The study quality was also assessed using the Biomarker Toolkit Assessment, which includes a 14-point checklist in four categories: Category 1 (rationale) with details about the burden of disease; Category 2 (analytical validity) covering experimental design, biospecimen quality, and quality assurance; Category 3 (clinical validity) assessing sensitivity/specificity, method validation, eligibility criteria of the population, and statistical analysis; and Category 4 (clinical utility) considering human factors, ethical approval, regulatory authorities, cost-effectiveness, feasibility of implementation, and utility in clinical care (**Table S4**) [6]. Each study was scored (yes: 2, unclear: 1, no: 0), with total scores of 1–9 indicating low quality, 10–18 moderate quality, and 19–28 high quality. Disagreements regarding study eligibility were resolved through discussion and consensus.

Data extraction and synthesis of results

Data extraction was conducted using EndNote v21.3 and Microsoft Excel 365 v2406. The reviewers organised the data into variables aligned with the review's aims, including author(s), publication year, first author's country, study design, sample size, genes, investigated molecules, roles of the genes in OC, and RoB and biomarker study quality assessments. The study design for each study was defined based on criteria established in the literature (**Table S5**). Outcomes data covered major findings directly associated with these genes, such as (1) signalling pathways in ovarian carcinogenesis and progression, (2) associations with OC risk factors, (3) prognostic performance, (4) links to metastasis, (5) clinicopathological characteristics, and (6) potential as biomarkers for predicting treatment response. Relevant data were organised into summary tables using Excel 365 v2406, and study characteristics were visualised. Studies were grouped by similarities in genes of interest and underlying mechanisms (**Table S6**).

QUANTIFICATION AND STATISTICAL ANALYSIS

No quantitative analysis was performed in the systematic review. Based on the 27 studies reviewed, calculations for generating a sunburst chart illustrating cancer hallmarks, based on the number of gene-related traits and study count, are available in **Table S7**. In the bioinformatic analysis, all quantitative and statistical analyses were conducted using various web-based tools to validate PLAT-M8-related gene

expression. These tools provided Gene Set Enrichment (GSE) analyses and data from The Cancer Genome Atlas (TCGA) for OC tumour tissues. First, the TNMplot tool compared differential gene expression in tumour, normal, and metastatic tissues [1]. Second, the Cancer Hallmark tool summarised commonalities in malignant transformation and progression using data from 6,763 genes [2]. Third, the Kaplan-Meier (KM) plotter website provided gene mRNA and RNA-seq expression data, and was used to assess the prognostic performance of genes for overall survival (OS) and progression-free survival (PFS) [3]. KM-plotter analyses were performed using median cutoff (**Table S8** and **S9**) for overall cases and subgroups based on chemotherapy, surgery type, stage, and histological types.

References

1. Bartha, Á. and Györfy, B. (2021) TNMplot.com: A Web Tool for the Comparison of Gene Expression in Normal, Tumor and Metastatic Tissues. *Int J Mol Sci* 22,
2. Menyhart, O. et al. (2024) A gene set enrichment analysis for the cancer hallmarks. *Journal of Pharmaceutical Analysis*, 101065
3. Györfy, B. (2023) Discovery and ranking of the most robust prognostic biomarkers in serous ovarian cancer. *Geroscience* 45, 1889-1898
4. Page, M.J. et al. (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 372, n71
5. Higgins, J.P.T. et al. (2011) The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 343, d5928
6. Savva, K.V. et al. (2023) The Biomarker Toolkit - an evidence-based guideline to predict cancer biomarker success and guide development. *BMC Med* 21, 383
7. Flanagan, J.M. et al. (2017) Platinum-Based Chemotherapy Induces Methylation Changes in Blood DNA Associated with Overall Survival in Patients with Ovarian Cancer. *Clinical Cancer Research* 23, 2213-2222