

## Transforming the Future of Treatment for Ovarian Cancer

Ying Dong<sup>1,2</sup>, Jyotsna Batra<sup>1</sup>, Kamal Anand<sup>3</sup>, Sharmila Bapat<sup>3</sup> and Judith A Clements<sup>1,2</sup>

<sup>1</sup>Cancer Program, Institute of Health and Biomedical Innovation, Translational Research Institute, Faculty of Health, Queensland University of Technology, Woolloongabba, QLD 4012, Australia

<sup>2</sup>Wesley Research Institute, Auchenflower, QLD 4066, Australia

<sup>3</sup>National Centre for Cell Science, Pune University, Ganeshkhind, Pune 411 007, India

\*Corresponding author: Ying Dong, Institute of Health & Biomedical Innovation (IHBI), Translational Research Institute (TRI), Faculty of Health, Queensland University of Technology 37 Kent Street, Woolloongabba, QLD 4102, QLD Australia, Tel: 61734437338; E-mail: [y.dong@qut.edu.au](mailto:y.dong@qut.edu.au)

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### Abstract

As for many other cancers, metastasis is the leading cause of death of patients with ovarian cancer. Vigorous basic and clinical research is being performed to initiate more efficacious treatment strategies to improve the poor outcome of women with this cancer. Current treatment for ovarian cancer includes advanced cyto-reductive surgery and traditional platinum and taxane combined chemotherapy. Clinical trials using novel cytotoxic reagents and tyrosine kinase inhibitors have also been progressing. In parallel, the application of robust unbiased high throughput research platforms using transcriptomic and proteomic approaches has identified that not only individual cell signalling pathways, but a network of molecular pathways, play an important role in the biology of ovarian cancer. Furthermore, intensive genomic and epigenetic analyses have also revealed single nucleotide polymorphisms associated with risk and/or aetiology of this cancer including patient response to treatment. Taken together, these approaches, that are advancing our understanding, will have an impact on the generation of new therapeutic approaches and strategies for improving the outcome and quality of life of patients with ovarian cancer in the near future.

**Keywords:** Ovarian cancer; Metastasis; Chemoresistance; Targeted therapy; Pharmacogenomics

### Introduction

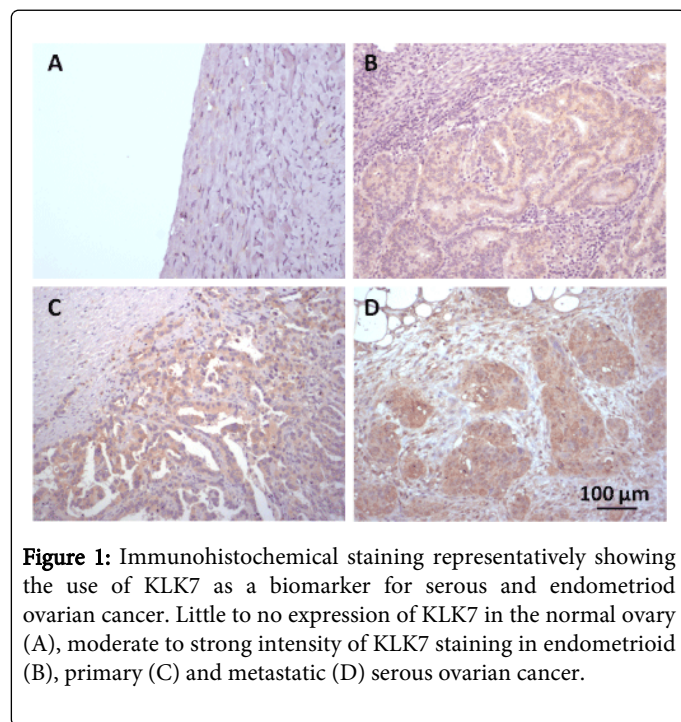
Malignant tumors have become a severe burden on the health system of numerous countries, with treatment resistant metastasis, the leading cause of death of patients including women with ovarian cancer [1,2]. Ovarian cancer remains the leading cause of death from all gynaecological tumors due to the lack of both symptoms at an early stage and a reliable clinical test. The majority (85-90%) of ovarian cancer is classified as epithelial histology and is the most aggressive form of this cancer. The dismal fact is that 75% of patients are diagnosed when the tumor has spread or metastasized into the peritoneal wall or abdominal cavity. Clinically, it has been a challenge to surgically remove all of the tumors even with advanced aggressive cyto-reductive approaches, and therefore chemotherapy with cytotoxic reagents is routinely administered following surgery. Most women respond to the chemo-treatment initially but the tumor will become resistant eventually leading to relapse; and this recurrent and chemo-resistant disease is the main cause of patient deaths. Over the past three decades, efforts have been made to overcome resistance to the front line platinum-taxane regimen. For platinum-taxane refractory cases, other options (as discussed below) are available such as, Pegylated Liposomal Doxorubicin (PLD), gemcitabine, the topoisomerase inhibitors, topotecan and etoposide, or a hormonal regimen with combined tamoxifen and anastrozole to reduce estrogen levels in the body [3]. However, the relapsed tumor cellular response rate to each drug eventually decreases leading to patient death. The development and clinical application of these drugs needs reliable preclinical predictive models to determine their therapeutic efficacy in

advance. In addition, current cancer research has revealed that the response of tumor cells to certain treatments varies in individuals as their molecular phenotypes and tumor microenvironments differ [4,5]. For this reason, numerous in vitro cell culture platforms have been established and utilised in preclinical studies to better mimic the tumor microenvironment seen in patients [6]. At present, cancer derived cell lines are the major tool for research and preclinical testing of the therapeutic potential of these new drugs. In this review, we wish to summarise the current understanding, novel therapeutic agents and approaches to ovarian cancer treatment, to lay a foundation for our further research leading to new treatment for this disease.

### Ovarian Cancer Pathogenesis

As for many other cancers, ovarian cancer is now recognised to have a heterogeneous nature as we have achieved a better understanding of the origin, development and progression of this cancer over the past 10 years. Conventional pathology has divided epithelial ovarian cancer into endometrioid, serous, clear cell and mucinous subtypes according to its histological phenotypes with many different immunohistochemical markers for the different subtypes being described (Figure 1) [7]. Traditionally, epithelial ovarian cancer is believed to derive from a single layer of flat or cuboidal mesothelial cells covering the ovary. Recent pathological studies have shown that ovarian cancer may arise from other organs, such as, high grade serous cancer from the fallopian tube after seeding in the ovary [8-11]. However, the site of origin cannot be determined from the pathology alone and increasingly is relying on molecular phenotypes to assist in this regard. Recent studies on combined clinical, molecular and genetic characteristics have proposed a two pathway model for this cancer: Type I and Type II tumors [12]. Type I tumors display low-

grade nuclear and architectural features, slow growth, and are associated with well-defined benign and borderline (low malignant potential) precursor lesions. Type II tumors comprise almost all of high grade serous epithelial ovarian cancer, with a high growth rate, metastasis and a less than 30% 5-year patient survival rate [13]. Furthermore, we now have a better understanding that due to the lack of anatomical barrier around the ovary, ovarian cancer cells are shed from the primary tumor site into the abdominal cavity as single cells or clusters that are floating in the peritoneal fluid [14]. These individual ovarian cancer cells and clusters adhere to the peritoneal membrane, invading into the underlining extracellular matrices and grow as secondary tumors. The metastasized tumors block lymphatic drainage leading to accumulation of fluid in the abdominal cavity, called ascites. In this scenario, patients have both solid tumors in the stroma and ovarian cancer cells/clusters floating in the ascites fluid as well. This clinical feature has been associated with poor outcome for women with this cancer, in particular the aggressive high grade serous histotype Type II tumors. While the underlining molecular mechanisms involved in these processes are still a major focus of ongoing research into metastatic spread, epigenetic association studies also have been in progress.



**Figure 1:** Immunohistochemical staining representatively showing the use of KLK7 as a biomarker for serous and endometrioid ovarian cancer. Little to no expression of KLK7 in the normal ovary (A), moderate to strong intensity of KLK7 staining in endometrioid (B), primary (C) and metastatic (D) serous ovarian cancer.

## Genetics Aspect of Ovarian Cancer

To unravel the complex nature of ovarian cancer biology, numerous studies have been carried out to search for genetic risk factors and to characterize molecular phenotypes of this tumor. For example, women carrying inherited mutations of BRCA1 and/or BRCA2 have increased risk in developing ovarian as well as breast cancers [2]. In Type I tumors, KRAS and BRAF mutations that activate the Mitogen-Activated Protein Kinase (MAPK) signaling pathway are frequent [15-17]. Mutation of the gene for Phosphoinositide 3-Kinase (PI3K) inducing activation of the PI3K/AKT/mTOR signaling pathway has also been reported in Type I tumors [15-17]. On the other hand, mutations of TP53, CCNE1 and chromosome instability are found in more than 80% of cases with Type II tumors [11,18]. In these tumors,

integrated genomic analyses have demonstrated different gene signatures which have subdivided the high grade serous epithelial ovarian cancers into differentiated, immunoreactive, mesenchymal and proliferative sub-groups [19]. In addition, regulation of ovarian cancer progression by microRNAs has revealed a cohort of potential biomarkers for diagnosis and monitoring of this disease [20]. The heterogeneous nature of ovarian cancer also makes it vital to identify susceptible risk loci, which may be able to attribute its many different sub-types to a reasonably distinct molecular basis, and also to establish molecular signatures which can aid in its diagnosis and prognosis.

## Single Nucleotide Polymorphisms (SNP) in Ovarian Cancer

Single Nucleotide Polymorphisms (SNPs) have been implicated in instances of drug resistance during treatment, mostly due to the expression and regulation variability they bring in individual genomes [21]. This makes the knowledge derived from genetic association studies valuable and clinically relevant for personalised medicine initiatives in future ovarian cancer treatment. Until the last decade, genetic association studies have been mainly undertaken using candidate gene approaches, which are relatively cheap and quick to perform and are focused on the selection of genes that have been related to the disease previously and thus are based on previous findings and require prior knowledge about gene function. Many researchers have undertaken the candidate gene study approach and identified risk alleles. An in depth analysis of recent ovarian cancer research reviewed by Braem et al., attribute genetic pathways of DNA repair, cell cycle, sex steroid hormone and oncogenic pathways of most relevance in assessing risk susceptibility [22] when observed through a priori gene studies. A few examples of candidate genes and corresponding genetic variants most successful in ascertaining a statistically significant association with ovarian cancer, in the order of the affiliated pathways, are as follows.

A SNP, rs11226, in RAD52 has been reported to be a significant ovarian cancer susceptible locus among DNA repair genes, as shown by studies from Auranen et al. [23], Quaye et al. [24], and Beesley et al. [25]. SNP rs2854344 in the RB1 gene, one of the cell cycle genes, showed a high risk association in two studies conducted in 2006 [26] and 2008 [27]. The validity of the Androgen Receptor (AR) gene as a sex steroid hormone receptor involved in ovarian cancer susceptibility has been shown in various studies (mentioned in [22]). SNP rs523349 in the SRD5A2 gene also been shown as a significant variant [25], among other hormone regulating genes. Another involvement of steroid hormones were seen by the up regulation of Kallikrein (KLK)10 expression by estrogen and estrogen plus progesterone treatment in aggressive ovarian cancers, followed by discovery of functional KLK10 SNPs in that loci [28]. Among the kallikrein genes, another instance of oncogenic involvement was noted in ovarian cancer survival with the SNP (rs266851) disrupting KLK15 splice variant [29]. Ratner et al., in an exploration study followed by a validation study [30], formed links between a KRAS oncogene with a variant allele harbouring SNP rs61764370 which disrupted the let-7 miRNA binding site on the gene. Another example of probable and significant risk allele is in the FGF2 gene (rs308447), uncovered by Johnatty et al. in 2009 [31] as a part of the Ovarian Cancer Association Consortium (OCAC). A recent study carried out to link the VEGF gene variants to ovarian cancer demonstrated the association of rs833068 with poor survival rates in Australian woman [32].

Albeit that there are several advantages of candidate gene association studies, they have also been criticised on various other

accounts due to non-replication of the results. Considering these aspects along with cumulative effect of multiple loci, and also complex disease heterogeneity, a fine tuning of the candidate gene approach in the future has been highly recommended [22,33]. The last decade has specifically seen a success in identifying ovarian cancer loci using Genome Wide Approaches (GWAS). This research has been driven by technological advances including mainly chip based technology and availability of robotics, that enabled very high throughput DNA scanning approaches in very large cohorts through large collaborations. GWAS included that conducted by the OCAC consortium have identified 14 independent risk loci for ovarian cancer

imparting low- moderate risk. These loci are summarised in Table 1 derived from the NHGRI GWAS catalogue (<http://www.genome.gov/GWASStudySNPS.cfm?id=7213>). Some of these loci are specifically associated with sub-phenotypes of ovarian cancer. The clinical use of these GWAS risk loci as early disease biomarkers is still not clear as each one of them imparts only moderate effects. These SNPs in combination along with high risk loci e.g. BRCA1/2 might have better predictive values. These large efforts however have identified many novel biological pathways and candidates for future targeted drug therapies and are expected to evolve further with the advent of new technologies including next generation sequencing.

SNPs	Sample Size (Cases, Controls)	Region	Mapped gene	Risk Allele Frequency	p-Value	OR or beta	95% CI (text)	References
rs3814113	3,769, 4,396	9p22.2	BNC2 - RPL31P42	0.68	4E-29 (All invasive)	1.21	[1.17-1.25]	
rs3814113	3,769, 4,396	9p22.2	BNC2 - RPL31P42	0.68	4E-32 (Serous invasive)	1.28	[1.23-1.33]	[96]
rs10088218	3,769, 4,396	8q24.21	MIR1208 - MIR3686	0.87	3E-12 (All invasive)	1.18	[1.13-1.24]	[96]
rs10088218	3,769, 4,396	8q24.21	MIR1208 - MIR3686	0.87	1E-17 (Serous invasive)	1.29	[1.21-1.36]	[96]
rs2072590	3,769, 4,396	2q31.1	HOXD-AS1	0.32	5E-11 (All invasive)	1.11	[1.08-1.15]	[96]
rs2072590	3,769, 4,396	2q31.1	HOXD-AS1	0.32	3E-10 (Serous invasive)	1.13	[1.09-1.18]	[96]
rs7651446	3,769, 4,396	3q25.31	TIPARP	0.05	2E-29 (All invasive)	1.44	[1.35-1.54]	[96]
rs7651446	3,769, 4,396	3q25.31	TIPARP	0.05	2E-34 (Serous invasive)	1.59	[1.48-1.71]	[96]
rs8170	3,769, 4,396	19p13.11	BABAM1	0.19	2E-7 (All invasive)	1.11	[1.07-1.15]	[96]
rs8170	3,769, 4,396	19p13.11	BABAM1	0.19	3E-14 (Serous invasive)	1.19	[1.14-1.25]	[96]
rs9303542	3,769, 4,396	17q21.32	SKAP1	0.27	6E-9 (All invasive)	1.12	[1.08-1.16]	[96]
rs9303542	3,769, 4,396	17q21.32	SKAP1	0.27	3E-10 (Serous invasive)	1.14	[1.09-1.18]	[96]
rs11782652	3,769, 4,396	8q21.13	CHMP4C	0.07	6E-9 (All invasive)	1.19	[1.12-1.26]	[96]
rs11782652	3,769, 4,396	8q21.13	CHMP4C	0.07	7E-10 (Serous invasive)	1.24	[1.16-1.33]	[96]
rs1243180	3,769, 4,396	10p12.31	MLLT10	0.31	2E-8 (All invasive)	1.10	[1.06-1.13]	[96]
rs1243180	3,769, 4,396	10p12.31	MLLT10	0.31	1E-7 (Serous invasive)	1.11	[1.07-1.15]	[96]
rs757210	3,769, 4,396	17q12	HNF1B	0.37	8E-10 (Serous invasive)	1.12	[1.08-1.17]	[96]
rs8170	1,768, 2,353	19p13.11	BABAM1	NR	4E-6 (Susceptibility)	1.12	[1.07-1.17]	
rs2363956	1,768, 2,353	19p13.11	ANKLE1	NR	1E-7 (Susceptibility)	1.10	[1.06-1.15]	
rs2072590	1,768, 2,354	2q31.1	HOXD-AS1	NR	5E-14	1.16	[1.12-1.21]	
rs2665390	1,768, 2,354	3q25.31	TIPARP	NR	3E-7	1.19	[1.11-1.27]	[97]
rs10088218	1,768, 2,354	8q24.21	MIR1208 - MIR3686	NR	3E-9	1.19	[1.12-1.25]	[97]
rs9303542	1,768, 2,354	17q21.32	SKAP1	NR	1E-6	1.11	[1.06-1.16]	[97]
rs7521902	1,768, 2,354	1p36.12	WNT4 - MIR4418	NR	5E-6	1.12	[1.07-1.18]	[97]

rs12794435	1,768, 2,354	11p14.3	LUZP2 - RPL36AP40	NR	5E-6	1.16	[1.09-1.23]	[97]
rs2084881	1,768, 2,354	17q21.32	SKAP1	NR	2E-6	1.12	[1.07-1.18]	[97]
rs3814113	1,817, 2,353	9p22.2	BNC2 - RPL31P42	0.68	5E-19	1.22	[1.16-1.27]	

**Table 1:** GWAS of ovarian cancer\*

\*Table modified from NHGRI catalogue.

## Epigenetic Therapies in Ovarian Cancer

Epigenetic regulation involves the effects mediated by reversible, inheritable influences in the transcriptome that are independent of any changes in the DNA sequence. In the normal homeostatic state, such regulation is responsible towards achieving a definite control over cell fate and determination [34]; various altered epigenetic mechanisms are associated with transformation. The most studied epigenetic modifications include DNA Methylation (DNMT), Histone Modifications (HDAC), miRNA regulation and nucleosome positioning. In the context of tumor progression global DNA hypomethylation of tumor cells compared to normal cells was the first example of association of epigenetic modifications [35]. Continuing research identified that hypermethylation of tumor suppressor genes that leads to their silencing and oncogene activation through hypomethylation play a significant role in tumor development [36,37]. Aberrant patterns of DNA methylation is a widespread phenomenon in progression of ovarian cancer with hypermethylation of BRCA1, RASSF1A, APC, p14ARF, p16INK4A, DAPK and MLH1 being most well studied [38,39]. The aberrant epigenetic signatures provided by these genes serve as predictive as well as prognostic biomarkers for evaluating the efficacy of epigenetic drugs.

The development of epigenetic therapies for cancer has targeted three critical components of epigenetic regulation viz. DNA methylation, histone modifications and post translational gene regulation by miRNAs. In ovarian cancer, epigenetic drugs have been effective in enhancing drug sensitivity of ovarian cancer cell lines and also *in vivo* experimentation mostly through re-sensitization of chemoresistant tumor cells to the action of conventional therapy [40]. A number of DNMT inhibitors (DNMTi, Table 2) and HDAC inhibitors (HDACi, Table 3) are currently progressing through clinical trials. Concurrently, miRNA targeting compounds, Histone Methyl Transferase and Histone Demethylase Inhibitors (HMTi and HDMi respectively) are currently being studied for possible preclinical efficacy.

DNMT inhibitors	Status of Clinical trial
SGI-110	Preclinical evaluation in progress
RG108	Preclinical evaluation in progress
SGI-1027	Preclinical evaluation in progress
MG98	Preclinical evaluation in progress
5-fluoro-2-deoxycytidine	Phase I
5-Azacytidine (Vidaza)	Completed; US FDA approved

Decitabine (Dacogen)	Completed; US FDA approved
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**Table 2:** DNA demethylating agents in clinical development

HDAC inhibitors	Status of Clinical trial
JNJ26481585	Preclinical
Dacinostat	Preclinical
Belinostat (PXD101)	Phase II
Resminostat (4SC-201)	Phase II
Panobinostat	Phase III
Etinostat	Phase II
Vornistat(SAHA); Zolina	Completed; US FDA approved (2007)
Romidepsin	Completed; US FDA 2009

**Table 3:** Histone deacetylase inhibitors in clinical development

## DNA demethylating agents in ovarian cancer

Despite the reported association of aberrant methylation in ovarian cancer, clinical experience of DNMTi is limited. The first compounds inhibiting DNA methylation approved by the FDA for the treatment of the disease were 5-Azacytidine (5-aza-C, Vidaza) and its deoxyribose analog, 5-Aza-2'-Deoxycytidine (5-aza-dC, Decitabine) [41,42]. Their effects have been attributed to activation of suppressed genes and thereby were directly related to reversal of epigenetic alterations [43,44]. DNMTi are believed to resensitize the chemoresistant tumors towards drugs [40]. In one study, treatment with decitabine re-expressed the DNA repair gene hMLH1 in platinum resistant A2780/CP70 ovarian cancer cells, and xenograft tumors derived from these cells were sensitized by decitabine to cisplatin, carboplatin, temozolomide, and epirubicin [45]. Phase II trials in ovarian cancer patients with methylated hMLH1 DNA in plasma was initiated with carboplatin alone or with decitabine to determine the role of the latter as a platinum sensitizer. However, this trial was prematurely terminated due to higher toxicity as compared with carboplatin monotherapy. Another phase II trial of thirty patients with platinum-resistant or -refractory ovarian cancer was conducted using a combination regimen involving azacitidine [46]. Most prominent side effects were myelosuppression, fatigue and nausea. Thereby, most early trials for epigenetic drugs were limited by toxicity, more particularly by myelosuppression [47]. More recently however, optimized lower doses of DNMTi like decitabine or 5-Aza-dC are suggested to be more effective in inducing DNA demethylation in a preclinical setup [48]. This has inspired the redesign of clinical trials with new regimens using DNMTIs either as single agents or in combination with other chemotherapeutic drugs [49], which could

pave the way for the future applications of these drugs in treatment of ovarian cancer.

### Histone deacetylase inhibitors in ovarian cancer

Acetylation and deacetylation of histones are well established permissive and repressive marks for gene expression respectively. These processes are mediated by specific enzymes described as Histone Acetyltransferases (HATs) and Histone Deacetylase (HDAC) respectively. HDACs and HATs regulate gene expression through chromatin remodeling during several normal developmental processes [50]. Certain alterations in the established dynamic equilibrium of the normal state can lead transformation. This provides the rationale for the use of HAT and HDAC inhibitors as cancer drugs [51]. Pan-HDAC inhibitors trichostatin A and butyric acid were reported to be effective in preclinical studies [52], but demonstrated limited clinical activity [53]. The first demonstrated clinically effective HDACi was Depsipeptide [54,55] that remains to be tested in ovarian cancer. Vorinostat (SAHA) is another HDACi and is a small molecule that binds directly in the active site of the enzyme in the presence of zinc. Oral route of administration and good bioavailability of Vorinostat is reported following treatment of cutaneous T-cell lymphoma [56]; it has progressed to phase II trials in non-selected, pretreated recurrent ovarian cancer patients, relapsing within 12 months after platinum based therapy [57]. Belinostat (PDX101) is currently in Phase II clinical trials in combination with cisplatin and carboplatin and exhibits increased tolerability and 31% objective response rate in patients [58]. Full exploration of the biological potential of these drugs can be realized only after there is a greater understanding of their molecular mechanism of action.

### New Cytotoxic Reagents

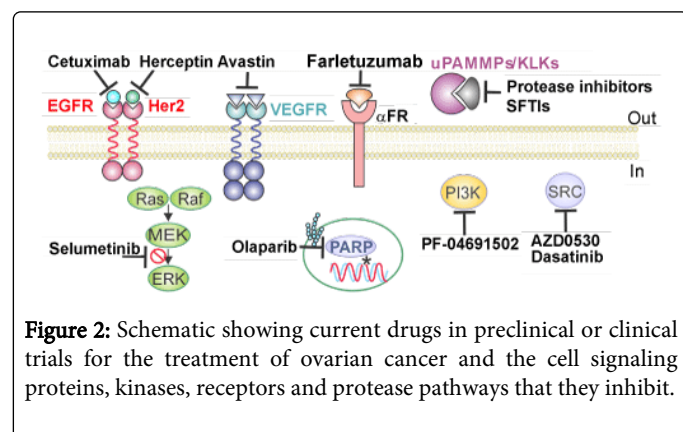
While research into the underlying molecular mechanisms of ovarian cancer metastasis is still in progress, progressive resistance to treatment remains the main challenge to cure this cancer. To treat patients with chemo-resistant recurrent ovarian cancer, novel cytotoxic agents have been generated and trialled [59]. These reagents include a marine-derived alkaloid trabectedin, a microtubule stabilizing agent patupilone and a glutathione analog, canfosfamide. Combined data analysis from 3 phase-II clinical trials on ovarian cancer patients with recurrence after carboplatin and/or paclitaxel treatment demonstrated that trabectedin is an efficacious single agent compared to the standard chemo-treatments especially for platinum sensitive patients [60]. A randomised phase II trial showed an improved progression free survival and an increased response rate on late relapsed patients using combined trabectedin and Pegylated Liposomal Doxorubicin (PLD) compared to PLD alone [61]. More specifically, a combined trabectedin and PLD regimen demonstrated an enhanced response in platinum sensitive patients [62], suggesting that it is a new chemo-treatment option for ovarian cancer in late relapse. However, patupilone failed to improve the outcome of relapsed patients [63]. A phase III study on canfosfamide showed a promising improvement of overall survival time over PLD suggesting its potential as a third line treatment for platinum-refractory or -resistant ovarian cancer [64].

### Molecular Signalling Pathway Targeted Therapies

Inhibition of activated signalling pathways, DNA repair enzymes and proteases has been vigorously investigated to treat ovarian cancer

with representative strategies as summarised in Figure 2. ErbB2 or Her2/neu is a member of the ErbB family of Receptor Tyrosine Kinases (RTKs) and a well-known target for efficacious treatment of breast cancer. Treatment with the Her2/neu neutralising antibody, Herceptin, has improved the outcome of women with Her2 positive breast cancer [65], but not significantly in ovarian cancer [3]. On the other hand, ErbB1 or EGFR, is over-expressed in 30-70% of ovarian cancer patients and associated with poor outcome [66]. A phase II trial showed that combined application of the EGFR inhibitor, cetuximab, with carboplatin and paclitaxel gave no prolonged progression free survival time compared to the conventional treatment [67]. However, the most exciting outcome is that two phase III trials using an inhibitor of the VEGFR and PDGF induced angiogenesis signalling pathway, Bevacizumab (Avastin), significantly prolonged disease free survival time in particular for women with advanced stage disease [68]. The folate transporter, αFR, is over-expressed in more than 90% of non-mucinous ovarian cancer and correlates with high tumor grade [69]. An extended phase II trial with relapsed platinum-sensitive ovarian cancer patients, using a monoclonal antibody farletuzumab to inhibit this pathway, showed a trend toward prolonged PFS [70]. Other potential molecular targets in ovarian cancer treatment involve Poly ADP-Ribose Polymerase (PARP), Src family members, Ras/Raf/MEK/MAPK pathway, JAK/STAT pathway and the PI3K/AKT/mTOR pathway [3,71,72]. For example, clinical trials with olaparib to inhibit PARP, Zibotentan to inhibit endothelinA, and a Src kinase inhibitor AZD0530 are still in progress [71]. Preclinical studies have shown their roles in reduced proliferation, migration, invasion, adhesion and chemoresistance using established cells lines in preclinical *in vitro* and *in vivo* animal xenograft models. Early research showed expression of Metalloproteases (MMPs) and their correlation with poor prognosis in ovarian cancer patients as reviewed [73]. Interestingly, expression of MMP2 in the tumor stroma is associated with a poor outcome in women with ovarian cancer [74,75]. However, clinical trials on MMP inhibitors alone or in combination with chemotherapy showed limited cytotoxicity in women with this cancer [76]. It has been acknowledged that the current inhibitors of MMPs have a broad spectrum and that specificity remains to be improved. In addition, new strategies have been proposed to design next generation approaches, such as using highly selective inhibitors or blocking antibodies against individual membrane-bound MMPs [73]. It has been long established that protein levels of Urokinase Plasminogen Activator (uPA), its receptors (uPAR) and endogenous inhibitors (PAI-1 and PAI-2) are associated with short progression free and overall survival time for ovarian cancer patients implying their therapeutic target potential [77,78]. Different strategies to inhibit uPA activity are under investigation [79] and an elegant delivery system using uPA-targeted nanobins has shown promise in reducing ovarian tumor burden in an *in vivo* mouse model [80]. Recent studies have also revealed aberrant expression of members of a serine protease family, Kallikreins (KLKs), in this cancer as reviewed [81], with simultaneous expression of several KLKs reported [78]. Activation between MMPs, uPA and KLKs via hydrolysis of their respective pro-forms has been reported implying a proteolytic network involving these protease families of enzymes [82,83]. Importantly, we are learning more of the roles of the KLK enzymes, using endogenous inhibitors and those recently designed by scientists and pharmaceutical experts. For example, Sunflower Trypsin Inhibitors (SFTIs) selectively blocked the activity of KLK4 [84] and KLK7 [85] in cancer cell based assays. We have reported that adding selective KLK4 SFTI-FCQR reduced multicellular aggregation in ovarian cancer SKOV3 cells and increased sensitivity to Taxol in a 3D-suspension

culture platform [86]. Currently these inhibitors are in preclinical studies using *in vitro* and *in vivo* animal models [87] which hopefully can be extrapolated to a clinical setting. Overall, numerous potential molecular therapeutic targets have been identified although we still face the challenge to test their potential in preclinical and clinical studies and identify which individual patients will benefit from a given drug.



**Figure 2:** Schematic showing current drugs in preclinical or clinical trials for the treatment of ovarian cancer and the cell signaling proteins, kinases, receptors and protease pathways that they inhibit.

## Integrated Platforms to Identify Therapeutic Targets for Individual Patients

Over the past 2 decades following completion of the human genome project and advancing technologies in transcriptome analysis, cancer patient datasets, with and without treatment, have been generated, such as in the The Cancer Genome Atlas (TCGA, <http://cancergenome.nih.gov/>) and Oncomine (<https://www.oncomine.org>) [88]. For example, transcriptome analysis has revealed chemo-response associated genes in ovarian cancer patient tissue samples, such as for platinum and taxane drugs [89,90]. In addition, proteomics platforms based on Mass Spectrometry (MS) and antibody based approaches, such as antibody microarray, have already provided critical tools to identify new targets for personalized treatment for cancer patients [91]. Moreover, platinum resistance associated targets have been discovered using the MS-based proteomics approach integrated with transcriptome analysis in ovarian cancer cells [92]. Furthermore, phosphoprotein and phosphopeptide enrichment strategies combined with quantitative MS have been used to discover active kinases [93]. A phase II clinical trial was carried out to evaluate the clinical benefit of vandetanib, a multi-kinase inhibitor of VEGFR, EGFR and RET, but the data showed that the daily monotherapy had no clinical benefit in women with recurrent ovarian cancer [94]. In this study, an antibody based reverse-phase protein lysate array was applied on paired tissue biopsies which detected both phosphorylated-(p)EGFR and pVEGF receptor-2, but only pEGFR was inhibited. The outcome of this study revealed that the remaining challenge for a successful treatment is that the identified biomarkers need to be validated in an *ex vivo* assay to determine the cellular response to the inhibitors using tissues/cells from patients. In this regard, numerous 3-Dimensional (3D) cell culture platforms to better replicate the tumor microenvironment akin to that seen in patients have been generated [6,95-98]. Overall, integrated strategies like these are needed to help choose the most efficacious treatment agents for individual patients.

## Future Direction in Ovarian Cancer Research and Treatment

In addition to the initial crucial cyto-reductive surgery, we still need efficacious therapeutic approaches to treat the remaining ovarian cancer cells and prevent tumor recurrence and metastases. Pharmacogenomic profiling will identify the molecular signalling network(s) of which inhibition will limit tumor growth, and also predict how to choose candidate agents. However, determination of which identified pathways to target as the therapeutic approach for individual patients remains a challenge to clinical oncologists. Application of tumor derived cells from patients in *ex vivo* assays may provide the key to predict the response for individual patients and a clearer direction to a more efficacious therapeutic approach. Alternatively, *in vivo* animal models with patient derived *ex vivo* tumor material may also be the tool to help identify the most efficacious therapeutic agents. In this way, the distance between our current understanding of the underlying biology of ovarian cancer progression and treatment resistance, and application of our knowledge in clinical management will be shortened. Importantly, we will be on the way to reach our ultimate goal that is to prolong the survival time and improve the quality of life of women with ovarian cancer.

## Conflict of Interest:

There is no conflict of interest to clarify.

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