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Ovarian Cancer: An Ever Challenging Malady

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Ovarian cancer is the fifth leading cause of cancer related deaths in women with a five year survival rate of only 30–40%. Amongst the three broad subgroups of ovarian cancer, epithelial ovarian cancer is the most common and is divided in mainly five subtypes based histology and clinical behaviour. In patients when the disease is still confined to ovaries, surgery alone is curative for more than 90% patients. Unfortunately, most women are diagnosed with advanced stage disease and recurs in majority despite of debulking surgery and initial response to chemotherapy. Thus ovarian cancer is still a challenge to clinicians which gets more complicated due to asymptomatic nature of the early stage disease and frequent development of resistance to standard therapies. Therefore, researchers worldwide are engaged in identifying markers for early detection of ovarian cancer, investigating molecular mechanisms of chemoresistance, improving detection methods and developing novel therapeutic measures. In this review, we attempt to discuss the contemporary research and challenges associated with epithelial ovarian cancer along with the future improvements in various areas such as early detection of ovarian cancer through Multiplex-Methylation specific PCR (MSP) assay and Serial Analysis of Gene expression (SAGE) assay and identifying new biomarkers, facilitating personalised chemotherapy regime by various chemo-response assays, novel drugs and targeted therapies which will aid in enhancing the overall survival rate in future and overcome this deadly gynaecologic disease.

INTRODUCTION

Ovarian cancer is a lethal cancer amongst the gynaecologic malignancies. Approximately 239,000 new cases are reported worldwide annually and around 152,000 women succumb to this fatal disease annually (GLOBOCAN, 2012). In India, ovarian cancer is the fourth most common cancer in women with an annual occurrence of 26,834 new cases (GLOBOCAN, 2012). Although majority of ovarian cancer incidence occur in post-menopausal women of 60–64 years, young women below the age of 20 often experience germ cell tumors, while borderline tumors are often presented in women in the median age of 30–40 years (Berek *et al.*, 2012). A higher incidence of ovarian cancer has been recorded in women with reproductive risk factors such as nulliparity, history of infertility, early menarche and late menopause. Multiparity and use of hormonal contraceptives are thought to act as a parapet against ovarian cancer (Negri *et al.*, 1991; Berek *et al.*, 2012).

At early stages, ovarian cancer is highly

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asymptomatic and therefore, remains undetected. Elevation of Cancer Antigen-125 (CA-125) level in blood and ultrasonography help to confirm presence of ovarian cancer (Rauh-Hain *et al.*, 2011). A combination of cytoreductive surgery and platinum based chemotherapy are used to thwart growth of tumor (Xiao *et al.*, 2012). However, often patients succumb to ovarian cancer due to recurrence of the disease (Perez *et al.*, 1993).

The significantly high relapse of ovarian cancer is attributed to acquirement of chemoresistance, thus preventing total elimination of ovarian cancer cells. Development of chemoresistance in cancerous cells is complex, and occurs due to several reasons including expression of beta-tubulin isotypes, over expression of P-glycoprotein (PGP) mediated expulsion of chemotherapeutic drugs, altered DNA repair mechanisms, increased drug detoxification, increased cell survival and decreased apoptosis (Gaikwad *et al.*, 2012; Ling, 2005). Chemoresistance acquired by tumor cells decreases the success of overcoming complete cure in ovarian cancer patients. Demonstration of differential chemoresponses indicates the need for personalized treatment regimens.

In the current review, we will highlight commonly used tumor markers and novel approaches towards early detection of ovarian cancer, multifactorial causes of chemoresistance, exploratory research towards development of chemoresponse assays and drugs currently in clinical trials to treat ovarian cancer efficaciously.

Ovarian cancer: A heterogeneous disease

The biggest challenge associated with ovarian cancer treatment is the enormous heterogeneity. The World Health Organization classification of ovarian tumors based on tissue of origin are as follows: surface epithelial-stromal tumors (65–70%), germ cell tumors (15–20%), sex cord stromal tumors (5–10%) and metastatic tumors (5%) (Berek *et al.*, 2010; Lee-Jones, 2004; Scully, 1987). Earlier notion of classifying serous (85%) (low and high grade), endometrioid (10–20%), mucinous (3–5%), clear cell (5–10%), Brenner tumors, transitional tumors and undifferentiated (< 1%) tumors as epithelial ovarian tumors is recently debated (Berek *et al.*, 2012; Lalwani *et al*., 2011; Kumran *et al.*, 2010). Since these subtypes show widely different clinicopathological features and behaviour, current classification categorizes ovarian cancer in two groups of Type I and Type II. Tumors that originate from epithelial lining of the ovary are clinically indolent and classified as Type I (includes low-grade micropapillary serous carcinoma, low-grade endometrioid, clear cell and mucinous carcinomas). Type I tumors grow slowly, usually from borderline tumors, present at stage 1a and show mutations in several oncogenes like *kras, braf, pten, arid1a, ppp2r1a* and *ctnnb1*. Tumors that are probably non-ovarian in origin but migrate to ovary often arise from the epithelium of fallopian tubes or through endometriosis and are grouped as Type II (includes high-grade serous carcinoma, high-grade endometrioid carcinoma, malignant mixed mesodermal

tumors and undifferentiated carcinomas). Type II tumors are present at advanced stages III and IV, aggressive in nature, exhibit mutations in *p53*, *brca1* and *brca2* (Kurman *et al.*, 2008). Type I tumors comprise of 20–30% of Epithelial Ovarian Cancer (EOC) (Bast *et al.*, 2009) while Type II tumors account for 70–80% cases (Colombo *et al.*, 2013).

Besides the histogenetic groups of ovarian tumors, the International Federation of Gynecology and Obstetrics (FIGO) have classified ovarian cancer in following stages:

Stage I: Growth limited to ovaries

IA - Growth limited to one ovary; no ascites present containing malignant cells. No tumor on the external surface; capsule intact

IB - Growth limited to both ovaries; no ascites present containing malignant cells. No tumor on the external surface; capsule intact

IC* - Tumor either stage IA or IB, but with tumor on surface of one or both ovaries, or with ascites present containing malignant cells, or with positive peritoneal washings

Stage II: Growth involving one or both ovaries with pelvic extension

IIA - Extension and/or metastases to the uterus and/or tubes

IIB - Extension to other pelvic tissues

IIC - Tumor either stage IIA or IIB, but with tumor on surface of one or both ovaries, or with capsule(s) ruptured, or with ascites present containing malignant cells, or with positive peritoneal washings.

Stage III: Tumor involving one or both ovaries with histologically confirmed peritoneal implants outside the pelvis. Superficial liver metastases equals stage III

IIIA - Tumor limited to the true pelvis, with negative nodes, but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces, or histologically proven extension to small bowel or mesentery.

IIIB - Tumor of one or both ovaries with histologically confirmed implants, peritoneal metastasis of abdominal peritoneal surfaces, none exceeding 2 cm in diameter; nodes are negative.

IIIC - Peritoneal metastasis beyond the pelvis > 2 cm in diameter and/or positive regional lymph nodes.

Stage IV: Growth involving one or both ovaries with distant metastases

If pleural effusion is present, there must be positive cytology to allot a case to stage IV. Parenchymal liver metastasis equals stage IV.

*In order to evaluate the impact on prognosis of the different criteria for allotting cases to Stage IC or IIC, it would be of value to know if rupture of the capsule was spontaneous, or caused by the surgeon; and if the source of malignant cells detected was peritoneal washings, or ascites (Heintz *et al.*, 2006).

Early detection of Ovarian Cancer

A major hurdle associated with effective treatment of ovarian cancer is "Early Detection". A majority of women exhibit vague symptoms like altered bowel and bladder habits, abdominal pain and swelling,

dyspepsia, nausea, vomiting, unusual fatigue and weight changes that are often misinterpreted as normal changes during menopause or ageing, and are often not correlated to the presence of ovarian cancer (Bankhead *et al.*, 2005). Therefore, ovarian cancer remains asymptomatic in early stage and is frequently detected at advanced stages, III or IV (Lalwani *et al.*, 2011; Sankaranarayanan *et al.*, 2006). Hence, it is pertinent to detect ovarian cancer at an early stage in order to treat patients effectively and increase survival.

Standard ways of detecting ovarian cancer include: ELISA-based approach to identify tumor markers, transvaginal ultrasound, magnetic resonance imaging (MRI), and computed tomography scan (CT).

Tumor markers for detecting ovarian cancer

Unlike cervical cancer where detection of high risk human papilloma viruses and a Pap smear test screens for presence of malignancy, ovarian cancer lacks defined screening tests. Thus, there is a need for novel molecular approaches to detect ovarian cancer at early stages. Biomarkers are unique biomolecules found in bodily fluids like blood, urine, serum, as well as in tissues, that may directly correlate with the presence of malignant tumors (Husseinzadeh, 2011).

A specific glycoprotein, CA-125 or MUC16, is currently used in clinics as a biomarker to detect disease and examine success of chemotherapy in ovarian cancer patients. Although 60% cases of early stage ovarian cancer demonstrate an increase in CA-125, elevated levels are also seen in cancers of fallopian tube, endometrium, breast and lung. Hence, CA-125 is not highly specific to ovarian cancers (Husseinzadeh, 2011). Besides, CA-125 may also be elevated in many benign conditions such as endometriosis, tuberculosis, fibroids, pelvic inflammatory disease. Although, CA-125 is neither sensitive nor specific for ovarian malignancy, however, currently it is the only serum marker widely used for early detection of the disease.

Recently, it has been demonstrated that secreted glycoprotein human epididymis protein 4 (HE4) is expressed at higher levels by serous and endometrioid epithelial ovarian cancer cells and may be used as a candidate tumor marker for these tumors (Drapkin *et al.*, 2005). HE4 and CA-125 tests along with the menopausal status of the woman is used in calculating the risk of ovarian cancer, using the risk of ovarian malignancy algorithm (ROMA), often used as a supplement to the standard pre-surgical evaluation of an adnexal mass to further assess the likelihood of malignancy. In September 2011, the US Food and Drugs Administration (FDA) approved the use of HE4 in calculation for ROMA.

Consistent efforts to identify new and alternative markers for ovarian cancer are ongoing. However, sensitivity and specificity remain a challenge. A study by van Haaften-Day and colleagues showed a combination of biomarkers CA-125, OVX1, and M-CSF (Macrophage-Colony Stimulating Factor) enabled detection of 85% of the ovarian cancer, while CA-125 alone could identify

only 69% of the cancers (van Haaften-Day *et al.*, 2001). Another study demonstrated elevated mesothelin in urine in 42% and 75% of early stage and advanced stage ovarian cancer, respectively (Badgwell *et al.*, 2007), emphasizing further evaluation of urine mesothelin as a potential biomarker for early detection of ovarian cancer. Bikunin, a glycoprotein secreted by hepatocytes that inhibits metastasis may be used as a probable prognostic marker for ovarian cancer. In a pilot study of 327 ovarian cancer patients, Bikunin was elevated in patients with inferior quality of debulking tumor and exhibited poor response to chemotherapy, with a survival period of 26 months (Matsuzaki *et al.*, 2005).

Other tumor markers, such as osteopontin, human kallikreins, M-CSF, vascular endothelial growth factor (VEGF), leptin, prolactin were reported to be associated with ovarian cancer and need further investigation (Husseinzadeh, 2011).

MicroRNAs (miRNAs) are a class of 19–35 nucleotide long post-transcriptional regulators, involved in degradation of messenger RNA (mRNA), and thereby regulate protein translation, as also various physiological processes. These small RNA molecules have emerged as candidate biomarkers for various malignancies (Chen *et al.*, 2013). Numerous studies have reported that anomalous expression of miRNAs in epithelial ovarian cancer may possibly aid detection of ovarian cancer at earlier stages (Chen *et al.*, 2013). Lorio *et al*. (2007) conducted a genome-wide microRNA expression profiling in 15 normal and 69 malignant ovarian tissues. The significant analysis of microarrays (SAM) and partitioning around medoids (PAM) tool analysis, identified 39 miRNAs and 29 miRNAs, respectively, enabling sorting of normal versus tumor samples. The authors further reported four up-regulated miRNAs i.e, *miR-200a, miR-200b, miR-200c, miR-141* and 25 down-regulated miRNAs that include miR-140, miR-145 in ovarian cancers. Further evaluation of these miRNAs in different histological subtypes, demonstrated increased expression of *miR-200a, miR-200c* in serous, endometrioid and clear cell carcinomas; up-regulation of *miR-200b, miR-141* in endometrioid and serous subtypes; increased expression of *miR-203, miR-205, miR-23* in endometrioid type; down regulation of *miR-140, miR-199a, and miR-125b1* in serous, endometrioid, clear cell histotypes, as compared to normal ovarian tissue (Lorio *et al.*, 2007).

However, all these biomarkers have been proven to be suboptimal with limited sensitivity and specificity and high false-negative rate for detection and have not helped to decline mortality due to ovarian cancer. Hence, researchers are looking for novel approaches to detect ovarian cancer at early stages (Zhang *et al.*, 2013) which include MSP and SAGE assays.

Multiplex Methylation-specific PCR assay

Methylation of CpG islands in genes can cause deregulated expression, which precedes clinical manifestation of symptoms. In order to identify the status of methylation in circulating

DNA, a novel multiplex methylation-specific PCR (MSP) assay was designed. Caceres *et al.* (2004) used MSP assay on a cohort of 50 patients diagnosed with ovarian tumors or primary peritoneal tumors and 21 archival stage I tumors to analyse the status of hypermethylation of genes *brca1, rassf1a, p14arf, death-associated protein kinase (dapkinase)*. The study reported that 70 out of 71 tumors (37 of 38 stage I tumors and 33 stage III–IV tumors) showed hypermethylation in at least one of the genes (Ibanez de Caceres *et al.*, 2004). Studies have shown anomalous methylation pattern of circulating tumor DNA in serum of patients with tumors of prostrate, colon, lung and breast could be used as prognostic markers (Zhang *et al.*, 2013). Expression of CpG island hypermethylation of seven gene*s – apc*, *rassf1a*, *runx3, cdh1, tfpi2, sfrp5,* and *opcml* was studied in 202 epithelial ovarian cancer serum samples. The multiplex MSP assay has demonstrated 83% specificity, 82% sensitivity and 91% accuracy over CA-125 alone which showed 50%, specificity, 72% sensitivity and 89% accuracy, respectively for early diagnosis of ovarian cancer. Further investigation on status of hypermethylation, hypomethylation, and overall epigenetic changes in genes can lead to better diagnosis of ovarian cancer at earlier stages (Zhang *et al.*, 2013).

Serial analysis of gene expression assay

Dr. Victor Velculeses, in 1995, developed serial analysis of gene expression assay (SAGE) to identify specific mRNA transcripts in pathologic state. The assay determines expression of up-regulated or down-regulated genes in neoplasms, and differentiates histological subtypes based on gene expression. *flj12988, cldn3* and *folr1* are some candidate genes which have been identified in ovarian cancer through SAGE assay (Zhang *et al.*, 2011).

Ultrasonography

Transvaginal or transabdominal ultrasonography is the standard non-invasive imaging method used in clinic to detect presence of tumors in ovaries (Figure 1a, 1b). Van Nagell *et al*. (2000) analyzed the importance of transvaginal sonography (TVS) in 14,469 asymptomatic women who were either more than 50 years or above 25 years with familial history of ovarian cancer. Two hundred patients who showed absence of abnormality at first TVS were subjected to another scan after a year. While post-menopausal patients presented with tumor volume of more than 10 cm^3 and premenopausal patients bearing more than 20 cm^3 tumor volume were subjected to another TVS within 4–6 weeks. Finally, 180 patients with repetitive abnormal scans were recommended for surgical debulking of the tumor. Out of 14,289 patients (who initiallyshowed no abnormality on TVS) only four developed ovarian cancer. Thus this study reports TVS screening to have 98% specificity, 81% sensitivity with a positive predictive value (PPV) of 0.094 and a negative predictive value (NPV) of 0.999 (van Nagell *et al.*, 2000). Another study was conducted to assess the efficacy of TVS and CA-125 on a cohort of 312

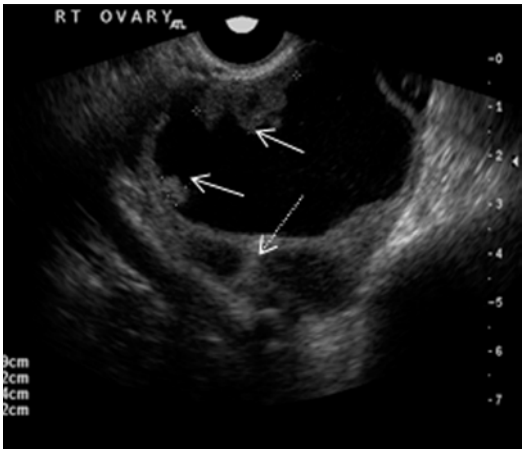

Figure 1a: Transvaginal ultrasonography showing a cystic adnexal mass with solid papillary nodules (solid arrows) and thick septations (dotted arrow), suggestive of neoplastic nature.

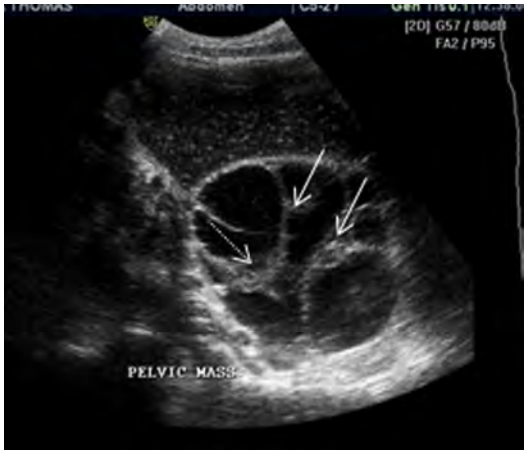

Figure 1b: Transabdominal ultrasonography showing a multicystic adnexal mass with thickened walls, thick septations (solid arrows) and solid areas in the centre (dotted arrow).

patients to identify women with high predisposition to ovarian cancer. The study showed TVS alone has a specificity, sensitivity, PPV and NPV of 90%, 40%, 6% and 99%, respectively, and CA-125 alone has a specificity, sensitivity, PPV and NPV of 96%, 60%, 13% and 99%, respectively. A combination of TVS and CA-125 showed better specificity and NPV, each at 99%, and PPV of 40% (Olivier *et al.*, 2006). The data indicated TVS as preferred mode of diagnosis for ovarian cancer despite limitations, which include (1) a 9.3% rate of PPV; (2) inability to differentiate benign from malignant tumors; and (3) ineffective in identifying cancerous cells in normal-sized ovaries (van Nagell *et al.*, 2000). An amalgamation of TVS and serum biomarkers will nonetheless accelerate early-stage detection of ovarian cancer in future (Fishman *et al.*, 2005). Currently, a large clinical trial involving more than 100,000 women is undergoing in UK, to understand the real potential of multimodal screening or MMS (TVS + CA125) against TVS alone. Though not complete yet, this trial indicates higher specificity in the MMS than in the TVS group resulting in lower rates of repeat testing and surgery (Menon *et al.*, 2009).

Computed tomography (CT)

Apart from ultrasonography, computed tomography (CT) scans also assist in diagnosis of ovarian cancer (Figure 2). Qayyum *et al.* (2005) have established that CT scans has 96% accuracy in identification of residual cells after surgery (Qayyum *et al.*, 2005). Another study demonstrated that CT scan has 87% precision in detection of benign or malignant tumors along with highspecificity (85%) and sensitivity (90%) and 55% and 89% accuracy in detecting stage I/II and stage III/IV, respectively (Byrom *et al.*, 2002).

Current treatment modalities

Advanced ovarian cancer is a Chemoresponsive but often not chemocurable

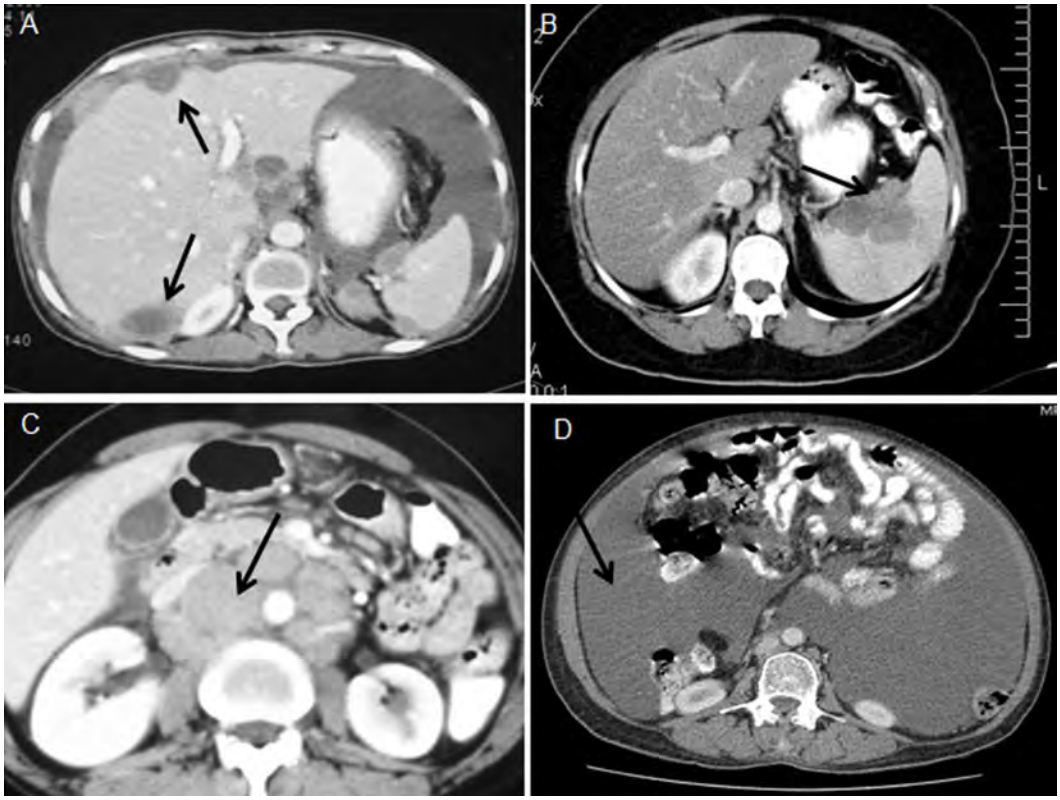

Figure 2: Contrast enhanced CT scans in advanced ovarian disease. **A)** Multiple liver surface deposits causing scalloping of the surface; **B)** Splenic hilar deposits; **C)** Multiple enhancing metastatic retroperitoneal nodes; **D)** Severe Ascites.

disease. Chemotherapy administered either intravenously (IV) or intraperitoneally (IP) is platinum-based combination of cisplatin or carboplatin and paclitaxel (Bast, 2011). Cisplatin kills cells by forming inter- and intra-strand DNA adducts via binding to N3 site on purine bases, stalling cell cycle at G2 phase and decreasing the ATP production in mitochondria. While paclitaxel prevents depolymerization of beta-tubulin subunits and blocks cell cycle at metaphase/anaphase of mitosis (Ling, 2005). Other drugs which have shown activity on ovarian tumors are methoxypolyethylene, PEGylated liposomal doxorubicin (PLD), topotecan, etoposide, tamoxifen, methotrexate, gemcitabine, vincristine, vinblastine, docetaxel and vinorelbine (Bookman *et al.*, 2009; Berek *et al.*, 2010).

The cornerstone of ovarian cancer treatment has been surgical removal of tumor followed by adjuvant chemotherapy. Sometimes surgical removal of tumor is difficult due to the extent of the disease. The choice of treatment in such cases is neoadjuvant chemotherapy (NACT) prior to optimal tumor debulking followed with additional chemotherapy (Robinson *et al.*, 2008). Chemotherapeutic drugs are usually administered IV, while ovarian cancer patients who have undergone optimal debulking surgery also have an option of IP

chemotherapy via an IP access port placed at surgery (Robinson *et al.*, 2008). IP chemotherapy has been reported as more than 10-fold effective than IV chemotherapy after surgical debulking and increases overall survival (OS) to 16 months (Bast, 2011). The combinatorial chemotherapy of IV/IP alleviates a median progression-free survival (PFS) of up to 16–21 months and median overall survival from 24–60 months. However, IP therapy remains to be accepted universally due to the adverse side effects like neurotoxicity and increased fatigue. Even with recent advances in treatment modalities, about 60% patients succumb to the disease within five years, which is attributed to relapse and acquired resistance to chemotherapeutic drugs (Armstrong *et al.*, 2006; Bast, 2011; Bookman *et al.*, 2009). Hence, the need of understanding the molecular basis of chemoresistance and relapse is crucial.

Chemoresistance in ovarian cancer

Chemoresistance is a phenomenon wherein a patient stops responding to the administered chemotherapeutic drugs, causing aggressive metastases and death (Figure 3). The patient may be intrinsically resistant or may acquire resistance to chemotherapy on successive exposures. Inability to mitigate and counter chemotherapy failure is attributed to several factors as elaborated.

Aberrant membrane transporters

Chemotherapeutic drugs are structurally diverse and have dissimilar intracellular targets. The entry–exit in a cell is dependent on transmembrane unidirectional influx and efflux pumps such as ATP-binding cassette (ABC) super-family membrane transporters (Nooter *et al.*, 1991). The ABC super-family membrane transporters consist of 48 genes and are subdivided into eight groups from ABCA to ABCG. The ABC proteins like PGP and multidrug resistance proteins like MDR-associated protein 1, breast cancer resistance protein (BCRP), lung resistance protein (LRP), expedite efflux of chemotherapeutic drugs and hinder accumulation of drugs inside cancer cells (Goff *et al.*, 2001; Ling, 2005).

MDR associated proteins (MRP), first discovered by Cole *et al*. (1992) are transmembrane proteins with a role in the efflux of accumulated drugs from the cells (Goff *et al.*, 2001). There are seven types of MRPs (MRP1MRP7) and each transports drugs in different capacities. MRP1 exhibits poor transport of paclitaxel than drugs conjugated to sulphate, glutathione. Overexpression of MRP2 facilitates removal of cisplatin, etoposide, doxorubicin, epirubicin, mitoxantrone and methotrexate (Borst *et al.*, 2000). MRP3, MRP4, and MRP5 expedite efflux of chemotherapeutic drugs like etoposide and gemcitabine (Hagmann *et al.*, 2010).

Platinum drugs are extremely polar compounds that do not enter a cell through passive diffusion, rather depend on active uptake via membrane associated copper transporters – hCTR1 and hCTR2 (Holzer *et al.*, 2004). Studies in yeast and mammalian cells showed that absence of CTR1 protein hinders platinum containing drug uptake

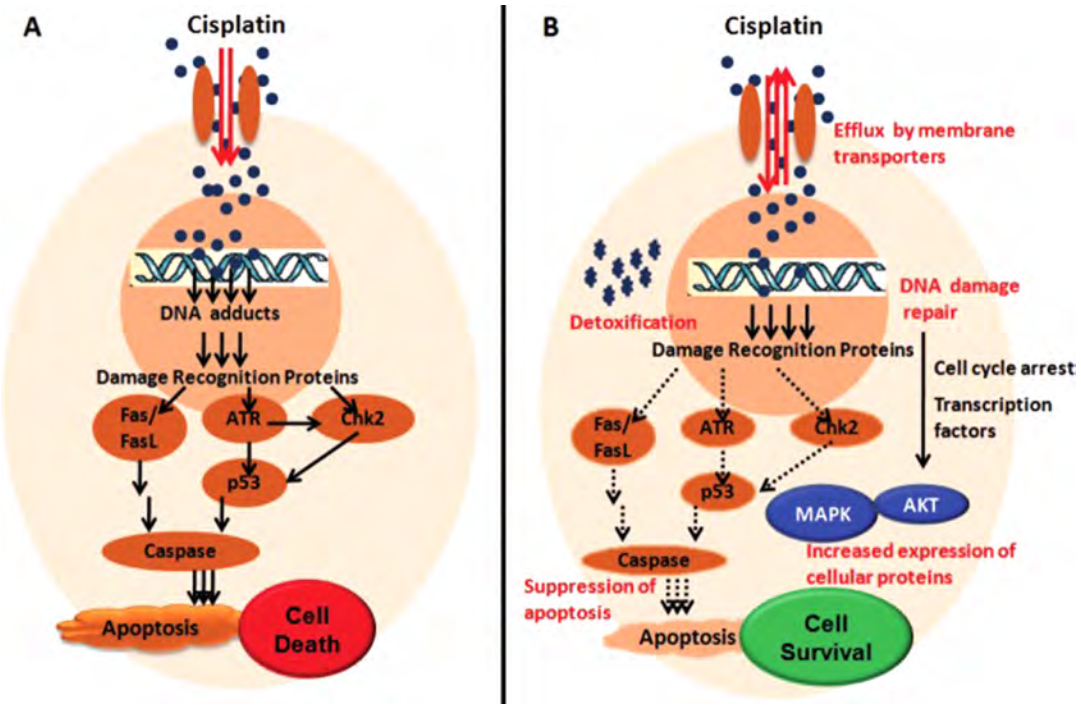

Figure 3: Schematic representation of **A:** Mode of action of platinum-based drug cisplatin: Cisplatin enters cell through membrane transporters and forms DNA adducts in the nucleus resulting in activation of DNA damage recognition proteins, which causes cell cycle arrest. Apoptotic machinery is further activated with continuous exposure to cisplatin leading to cell death (arrows in black); **B:** Mechanisms of acquired resistance to cisplatin (text in red): Cisplatin entered in the resistant cells is either **i)** effluxed by up-regulation and overexpression of membrane transporters or **ii)** detoxified by the Glutathione transferases. Formation of lower level of DNA adducts results in the **(iii)** activation of DNA damage repair proteins and further global changes involved **iv)** increased expression of cellular proteins like transcription factors, MAPK and Akt etc., which leads to cell survival and also **v)** suppression of apoptosis eventually leading to chemoresistance. [Source: Gaikwad et al., 2012]

(Holzer *et al.*, 2006; Howell *et al.*, 2010). A study demonstrated that overexpression of hCTR1 in A2780 (ovarian epithelial cancer cell line) not only increased copper influx 13.7 fold but also improved intake of cisplatin by 55% after 24 hours (Holzer *et al.*, 2004). Sensitive ovarian cancer cell lines A2780, 2008 and IGROV-1 were more receptive to cisplatin than cisplatin-resistant A2780, 2008 and IGROV-1 cell lines. These cisplatin-resistant A2780, 2008 and IGROV-1 cells were found to be cross resistant to copper uptake, thus elucidating the role of human copper transporters in influx of platinum drugs apart from copper homeostasis (Katano *et al.*, 2002). Kamazawa *et al*. (2002) analyzed expression of MDR1, MRP1, MRP2 in SKOV-3 (p53-null cells), KOC7c, KF, paclitaxel-resistant KF (KFTx) ovarian carcinoma cell lines and in ovarian cancer patients with relapse after paclitaxel treatment. Increased resistance to paclitaxel and expression of drug resistance genes were noted in SKOV-3, KOC7c, and KFTx cell lines. In addition, 6 of 27 paclitaxel non-responder patients showed increased MDR1 expression (Kamazawa *et*

al., 2002). The study thus emphasized that expression of multidrug resistance genes correlates with higher resistance to paclitaxel.

Anti-oxidant protein 1 (ATOX1) transports circulating platinum drugs to specific organelles and regulates their discharge out of the cell via efflux pumps ATP7A and ATP7B (Howell *et al.*, 2010). ATP7A and ATP7B are P-type ATPase membrane transporters involved in maintaining homeostasis of heavy metals like cadmium, copper, and zinc (Nakayama *et al.*, 2002; Nakayama *et al.*, 2004). ATP7A is present in all the organs except liver, wherein the expression of ATP7B is predominant (Samimi *et al.*, 2004). Katano and colleagues demonstrated increased expression of ATP7A in cisplatin-resistant A2780 and 2008 ovarian cell, and an accrual in ATP7B expression in IGROV-1 cells (Katano *et al.*, 2002). Another study reported a 1.5-fold higher expression of ATP7A in the ovarian cell line, 2008 through transfection with ATP7A expression vector that showed minimal intake of copper and conferred resistance to cisplatin, oxaliplatin and carboplatin (Samimi *et al.*, 2004).

Increased expression of ATP7A was found in 18 of 54 treated ovarian carcinomas with poor survival (Samimi *et al.*, 2003). Expression of ATP7B, MDR1, MRP1, MRP2, LRP and BCRP was analyzed by real-time analysis in 82 ovarian cancer patients exposed to cisplatin-based chemotherapy after tumor debulking. Varied expression of genes [*atp7b* (43.9%), *mdr1* (24.4%), *mrp1* (86.6%), *mrp2* (81.7%), *lrp* (92.7%) and *bcrp* (53.7%)] were noted in the samples with significant expression of *atp7b* (p = 0.01) in relapsed cases, indicating *atp7b* as a strong candidate causing chemoresistance in cisplatin treated and relapsed ovarian cancer patients (Nakayama *et al.*, 2002).

In order to inhibit action of multidrug resistance proteins and achieve better efficacy of cisplatin treatment, several approaches including antisense technology, oligonucleotide combinatorial technology, small molecule inhibitor technology are in use. Several pharmaceutical companies are developing IV agents and oral compounds to block PGP (Persidis, 1999). However, toxicity and undesired inhibition of these transporters in normal organ are often an impediment in the clinical trials.

Altered drug metabolism

Another protective mechanism adopted by cells to escape deleterious effects of drugs is the glutathione-dependent detoxification mechanism. Like normal cells, cancer cells try to make drugs ineffective by upregulating the cellular proteins that expedite detoxification. Predominantly glutathione (GSH), glutathione S transferase (GSTs), glutathione peroxidase (GPx) and metallothioneins facilitate detoxification of toxins and drugs, and neutralize reactive oxygen species (Abdullah *et al.*, 2013; Ling, 2005). GSH homeostasis is important as GSH deficiency causes oxidative stress, while excess results in increased antioxidative ability leading to drug inactivity and propelling chemoresistance in tumors (Abdullah *et al.*, 2013; Syng-Ai *et al.*, 2004).

GSTs belong to a family of enzymes that facilitate coupling of glutathione to various molecules, including chemotherapeutic drugs. Functional polymorphism in 3 *gst* genes namely *gstm1, gstt1* and *gstp1* was associated with treatment and survival of a cohort of 215 primary epithelial ovarian cancer patients using PCR techniques such as PCR-RFLP. The study reported an increased progression of the disease in late-stage patients with higher *gstm1* compared to *gstm1* null patients, while no such association of *gstm1* with progression of disease in early-stage patients was noted (Saga *et al.*, 2008). Similarly patients without *gstm1* and decreased *gstp1* polymorphisms had 60% better progression free survival and 40% overall survival than patients with *gstm1* and *gstp1* polymorphisms (Beeghly *et al.*, 2006). Another study reported presence of GPX3 in KK, OVMANA, OVSAYO and RMG-1 (clear cell ovarian carcinoma cell lines) by DNA microarray and real-time PCR. These cells when transfected with shRNA against *GPX3* showed decreased level of *GPX3* expression with increased sensitivity to cisplatin (Saga *et al.*, 2008).

Apart from rapid efflux of drugs mediated by cellular detoxification mechanisms, elevation in expression of factors involved in repair of damaged DNA also confers chemoresistance in ovarian cancer.

Enhanced DNA repair mechanisms

DNA adducts formed in tumor cells on exposure to chemotherapeutic drugs activates various DNA repair mechanisms, including nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), non-homologous end-joining (NHEJ) and homologous end-joining (HR) pathways (Ling, 2005; Martin *et al.*, 2008). Enhanced rate of DNA repair results in chemotherapy failure.

NER pathway predominantly repairs cisplatincarboplatin invoked intrastrand and interstrand DNA adducts. DNA adduct is usually formed in a single strand that is recognized by excision repair cross complementation (ERCC1) protein. After removing the lesion, DNA polymerase uses undamaged single strand as a template to resynthesize complementary sequence and the ligase seals the nick to complete repair of DNA. Selvakumaran *et al.* (2003) demonstrated that NER facilitates cisplatin induced DNA damage in ovarian cancer cell lines A2780, OVCAR-4 and OVCAR10. Resistant cell lines of OVCAR10 and OVCAR-4 showed higher expression of ERCC1, and antisense RNA against ERCC1 converted cisplatin-resistant OVCAR10 cells to cisplatin-sensitive. The study demonstrated that cytotoxic action of cisplatin may be enhanced by altering expression of factors involved in NER pathway (Selvakumaran *et al.*, 2003).

Mismatch repair (MMR) pathway removes mismatched bases incorporated through insertion and deletion by DNA polymerase, and has often escaped proof-reading mechanisms. Three steps involved in MMR are initiation, excision and resynthesis that are regulated by several Mut proteins, viz., hMSH1, MLH1, MSH3, MSH6 and PMS2.

Effective removal of tumor cells is dependent on active MMR pathway. However, methylation of *hmlh1* gene resulting in inactivation of MMR, causes resistance to platinum drugs and consequent poor survival (Ling, 2005; Martin *et al.*, 2008; Richardson *et al.*, 2005). BER pathway removes non-bulky damaged DNA bases, abasic sites and DNA single strand breaks (SSBs) that occur on exposure to alkylating drugs and other chemotherapeutic drugs (Kinsella, 2009). Fishel *et al*. (2007) reported that combination of temozolomide and methoxyamine (BER pathway inhibitors) invoked higher cell death in ovarian cancer cell lines IGROV-1, OVCAR-3 and SKOV-3 (Fishel *et al.*, 2007). The study emphasized that chemotherapeutic drugs in combination with inhibitors of BER pathway may potentiate ovarian cancer treatment.

Numerous factors such as ionizing radiation, reactive oxygen species and genotoxic chemicals cause SSBs, which when left unrepaired may form double strand breaks (DSB) in the S-phase of the cell cycle, causing cell death. Homologous repair (HR) and NHEJ pathways ensure repairing DSB and prevents cells from dying. DSB repair pathways are mediated by numerous genes including: *brca1*, *brca2, atm, atr, rad50, mre11, nsb1* and *fanc.* Mutation in *brca1* and *brca2* has a 15–40% increased chance of being afflicted with ovarian cancer. Expression of BRCA1 and BRCA2 varies in histological subtypes of ovarian cancer as well (Cerbinskaite *et al.*, 2012). A study analyzed DNA repair related genes: *parp1, ercc1, xpa, xpf, xpg, brca1, fanca, fancc, fancd2,* and *fancf* in 77 stage I, 88 stage III and 13 borderline ovarian carcinomas by real-time analysis. Expression levels of ERCC1, XPA, XPF and XPG were higher in stage I than stage III samples, thus correlating with advanced stage of disease. Whereas, BRCA1, FANCA, FANCC, FANCD2, and FANCF were lower in borderline and stage I than stage III samples. Also, patients with highest level of ERCC1 and BRCA1 when treated with platinum based therapy demonstrated better progression free survival than those treated with a combination of platinum and taxol, thus, indicating a role for DNA repair genes in overall and progression free survival in ovarian cancer patients (Ganzinelli *et al.*, 2011). Although numerous studies are being conducted to decipher factors that contribute to chemoresistance, the need of the hour is to establish personalized chemotherapy regimes.

Chemoresponse assays

Several exploratory research projects have been undertaken to establish chemoresponse assays to predict PFS and OS, and measure sensitivity to particular chemotherapeutic drugs to limit unnecessary expenditure, and aid in establishing personalized treatment regimen (Rutherford *et al.*, 2013). Numerous chemo-response assays such as differential staining cytotoxicity assay (DiSC), extreme drug resistance assay (EDR), histoculture drug resistance assay (HDRA) and adenosine triphosphate (ATP) bioluminescence assay have been developed that share four common steps: (1) isolation of cells from tissue, *in vitro*

on medium or soft agar; (2) incubation of cells with several drugs at different concentrations; (3) inspection of cell survival fractions; and (4) analysis of obtained results.

A recent study used ChemoFX assay in a non-interventional, unbiased clinical trial on 262 ovarian cancer patients. The tumor samples were collected at time of recurrence and sent for *in vitro* analysis and simultaneously treatment regimens were initiated. Fifty five percent patients bore platinum-sensitive recurrent EOC where high grade papillary serous tumors were most abundant. Both single and dual agent combination chemotherapies to a maximum of four cycles were administered and 25-30% patients responded to the treatment. More than 50% of tumors were found to be responsive to minimum one drug tested *in vitro*, indicating that chemoresponse assay based informed personalized chemotherapy may benefit platinum-sensitive and platinum-resistant recurrent EOC patients (Rutherford *et al.*, 2013).

Molecular imaging modalities

Apart from using biomarkers and laproscopy, analysis of IP infiltration, non-invasive molecular imaging technologies like CT, magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), diffusion weighted imaging (DWI) are routinely used to determine the stages in ovarian cancer. Recently, a comparative study on imaging techniques (Doppler ultrasonography CT, PET/CT, MRI) on 132 ovarian cancer patients identified 95 malignant tumors, 13 borderline tumors and 25 benign tumors. The study highlighted PET/CT as a preferred technique as it showed higher sensitivity (91.6%), specificity (81.6%), PPV (92.6%), and NPV (79.5%) in detecting malignant tumors. Precision of PET/CT in detecting benign cases versus those that are borderline/malignant was higher than Doppler ultrasonography, MRI or CT (Nam *et al.*, 2010). Apart from using PET/CT combination, PET alone showed immense diagnostic potential to detect tumors in patients with indecisive transvaginal ultrasonography, presence of metastases and aid in staging of ovarian cancer (Musto *et al.*, 2011). Other than ^{18}F-FDG, molecules like 16-[18F] fluoro-17-estradiol (FES), ^{11}C-Choline, and ^{15}O-PET are actively used to assess ovarian tumors (Tsujikawa *et al.*, 2008).

Recent progress in imaging modalities is demonstrated by a novel method called optical coherence tomography (OCT) utilizing near-infrared as source of light for non-invasive diagnosis. Hariri and colleagues were the first to combine OCT with routine laproscopy (LOCT) to differentiate normal ovary, epithelial ovarian carcinoma, and endometriosis. Further, combination of OCT with ultrasound guided transvaginal imaging may pave way for less invasive methods to visualize uterine endometriosis (Hariri *et al.*, 2009).

Due to limitations with anatomic imaging through CT and MRI scans in identifying tumors, functional imaging is gaining prominence in gynecologic cancers

(Motoshima *et al.*, 2011). DWI is a non-invasive functional MRI method (DWMRI) that determines diffusion of water molecules in tumors, providing information on density, volume and size. Differences in cellularity of tumors also enables differentiating benign and malignant tumors (Motoshima *et al.*, 2011). DWMRI has immense potential in predicting cytoreductive success in patients diagnosed with advanced ovarian cancer with a sensitivity of 91.1%. DWMRI can facilitate visualization of solid tumors and malignant deposits by providing an increased contrast versus noise ratio (Espada *et al.*, 2013). Thus, DWI opens up new avenues to determine response of ovarian cancer patients to proposed treatments in real time.

Novel chemotherapeutic drugs

A major problem faced by ovarian cancer patients on successive exposure to platinum and taxol compounds is recurrence of tumor. In order to alleviate OS and PFS, various new drugs have been initiated in clinical trials. Epothilones, the metabolites produced by myxobacterium (*Sorangium cellulosum)* is under investigation in various clinical trials for cytotoxicity in cancer cells. Six types of water soluble epothilones (A to F) inhibit microtubule function by preventing depolymerization of microtubules, initiating cell cycle arrest at G2/M phase, similar in action to paclitaxel (Reichenbach *et al.*, 2008). Currently five epothilones (ZKEPO, ixabepilone, patupilone, KOS-862 and BMS-310705) are in clinical trials.

Trabectedin or Yondelis extracted from *Ecteinascidia turbinate* (a marine sea squirt) induces apoptosis by producing superoxides which cleave DNA strand and invoke cell cycle arrest. A combination therapy on 337 platinum-resistant ovarian cancer patients showed 6–12 months of platinum-free hiatus compared to 335 patients treated with only PEGylated liposomal doxorubicin (PLD) (Krasner *et al.*, 2012). Krasner *et al*. (2007) also conducted a study on response rate to trabectedin in platinum-sensitive or platinum-resistant recurrent ovarian cancer patients. Patients were subjected to weekly infusion of trabectedin for 3 hours for three consecutive weeks followed by a week of no treatment. Sixty two platinum-sensitive patients showed a PFS of 5 months versus 2 months PFS in 79 platinum-resistant cases, while overall response rate (ORR) was 29% and 6.9% in platinum-sensitive and platinum-resistant patients, respectively.

Canfosfamide also called as *telcyta TLK286* was evaluated in combination with PLD in 125 platinum-resistant ovarian cancer patients in a trial (NCT00350948). PFS of 5.6 months and 3.7 months were achieved in combination treatment and only PLD treatment, respectively. Moreover, there was a lower incidence of palmar-plantar-erythrodysesthesia in patients subjected to canfosfamide + PLD than PLD alone (23% versus 39%) (Vergote *et al.*, 2010). A phase III clinical trial on 247 platinum-resistant ovarian cancer patients evaluated efficacy of a combination of canfosfamide + carboplatin against liposomal doxorubicin. The authors reported an overall response rate (ORR) of

31.6% versus 10% in canfosfamide + carboplatin against liposomal doxorubicin treatment, respectively (Rose, 2007).

Targeted therapy for ovarian cancer

In contrast to breast cancer, targeted therapy is still not a standard practice of care for ovarian malignancy. Bevacizumab (Avastin) is an antiangiogenic humanized recombinant monoclonal antibody that inactivates VEGF and is thought to prevent VEGF-mediated cell growth in tumors. Efficacy of bevacizumab was tested in Gynecologic Oncology Group (GOG) protocol 218 (GOG 218), a phase III placebo-controlled clinical trial in a cohort of untreated 1873 advanced stage epithelial ovarian cancer, primary peritoneal and fallopian tube cancer patients. The study reported a median PFS of 14.1 months in patients who received concurrent and maintenance bevacizumab along with carboplatin + paclitaxel against 10.3 months in patients treated with carboplatin + paclitaxel. A multi-centric phase III clinical trial, ICON-7 (International Cooperative Group for Ovarian Neoplasia) studied effect of bevacizumab in 1528 stage IAIIA and stage IIBstage IV ovarian cancer patients. Patients on bevacizumab along with carboplatin + paclitaxel showed 19 months median PFS versus 17.3 months in control group. Bevacizumab efficacy was examined in 484 patients with recurrent ovarian cancer in a phase III clinical trial called OCEANS. Patients treated with 6–10 cycles of bevacizumab + carboplatin + gemcitabine and carboplatin + gemcitabine + placebo showed a median PFS of 12.4 months and 8.4 months, respectively. However, adverse effects such as hypertension, gastrointestinal perforation caused due to use of bevacizumab, were observed in patients in all clinical trials. Besides, incorporation of bevacizumab along with other chemotherapeutic drugs did not improve OS of women diagnosed with ovarian cancer. Thus, US FDA did not approve the use of bevacizumab as standard practice in the treatment of ovarian cancer (Eskander *et al.*, 2013).

Pazopanib (Votrient) prevents angiogenesis by inhibiting VEGF receptors (VEGFR1, VEGFR2, and VEGFR3), platelet-derived growth factor receptor (PDGFR), and C-Kit. Phase II clinical trial, is currently underway to measure efficacy of pazopanib in combination with topotecan on patients presenting with recurrent epithelial ovarian cancer, fallopian tube cancer and peritoneal cancer (NCT01600573). A drug called Olaparib (AZD2281) binds to poly (ADP-ribose) polymerase (PARP) and inhibits DNA repair mediated by PARP. A phase III clinical trial (NCT01844986) is underway to understand efficacy of Olaparib in ovarian cancer patients carrying *brca* mutation and treated with platinum-based chemotherapy.

Failure of chemotherapy with first line of platinum drugs has prompted investigations on establishing chemosensitive and chemoresistance assays to determine response of ovarian tumor to second-line chemotherapeutic drugs (Jordan *et al.*, 2013). Progress in chemoresponse assays will herald an era of personalized regimen of

chemotherapy that may benefit ovarian cancer patients. It is anticipated that translation of potential drugs from bench-to- bedside will not only improve OS rate and progression free survival but will also extend the current five-year survival rate.

Future directions

The 21st century has witnessed significant advances in diagnosis, therapy and disease management in ovarian cancer that has reduced the overall mortality rate. Ovarian cancer is not an exception as five-year survival rate has increased over the last 30 years, however, the final solution is still not in sight. The survival rate varies greatly according to how early the disease is diagnosed. Extensive research on identifying new tumor or serum-based biomarkers is in progress worldwide, and several promising candidates like HE4 are either in clinic or ready to enter the clinical trials. It is now obvious that not one but a combination of biomarkers will probably be the future choice after extensive validation in large cohorts, with advanced technologies and well designed assays.

Although early stage ovarian cancer patients have the potential to live a disease free life, women with advanced disease and recurrent disease require better treatment. Advanced imaging techniques combined with targeted therapy to tackle the tumor burden for optimal debulking surgery seems a thrust area. Many newer imaging modalities such as DWMRI, LOCT along with the standard PET/CT are being adapted in clinic. A quest for therapeutic molecules to target advance and subtype specific ovarian cancer is ongoing. For the relapse cases, the need is again on developing alternate therapeutic molecules based on detailed understanding of drug resistance of the cells. A focus on early detection of acquired chemoresistance needs to be actively pursued to alleviate the cytotoxic effects of platinum–taxol therapy. High-throughput genomic analyses, phage or antibody display techniques may add in identifying markers to detect patient population acquiring resistance towards the standard therapy. Globally, increased focus on various pathways to ovarian cancer and modalities towards early detection, better prognosis and management of the cancer patients is anticipated. The hope is to shift the paradigm for ovarian cancer from a more controlled chronic disease to an ultimate cure.

CONFLICT OF INTEREST

The authors claim no conflict of interest.

REFERENCES

Abdullah LN, Chow EK. Mechanisms of chemoresistance in cancer stem cells. *Clin Transl Med* 2013;2:3.

Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, *et al*. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *New Eng J Med* 2006;354:34-43.

Badgwell D, Lu Z, Cole L, Fritsche H, Atkinson EN, Somers E, *et al*. Urinary mesothelin provides greater sensitivity for early stage

ovarian cancer than serum mesothelin, urinary hCG free beta subunit and urinary hCG beta core fragment. *Gynecol Oncol* 2007;106:490-497.

Bankhead CR, Kehoe ST, Austoker J. Symptoms associated with diagnosis of ovarian cancer: a systematic review. *Br J Obstet Gynaecol* 2005;112:857-865.

Bast RC, Jr, Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. *Nat Rev Cancer* 2009;9:415-428.

Bast RC, Jr. Molecular approaches to personalizing management of ovarian cancer. *Ann Oncol* 2011;22(S8):viii5-viii15.

Beeghly A, Katsaros D, Chen H, Fracchioli S, Zhang Y, Massobrio M, *et al*. Glutathione S-transferase polymorphisms and ovarian cancer treatment and survival. *Gynecol Oncol* 2006;100:330-337.

Berek JS, Crum C, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum. *Int J Gynaecol Obstet* 2012;119(S2):S118-S129.

Berek JS, Hacker NF. Berek and Hacker's Gynecologic Oncology, 5th edn, Philadelphia, PA: Lippincott Williams and Wilkins, 2010.

Bookman MA, Brady MF, McGuire WP, Harper PG, Alberts DS, Friedlander M, *et al*. Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a Phase III Trial of the Gynecologic Cancer Intergroup. *J Clin Oncol* 2009;27:1419-1425.

Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. *J Natl Cancer Inst* 2000;92:1295-1302.

Byrom J, Widjaja E, Redman CW, Jones PW, Tebby S. Can pre-operative computed tomography predict resectability of ovarian carcinoma at primary laparotomy? *Br J Obstet Gynaecol* 2002;109:369-375.

Cerbinskaite A, Mukhopadhyay A, Plummer ER, Curtin NJ, Edmondson RJ. Defective homologous recombination in human cancers. *Cancer Treat Rev* 2012;38:89-100.

Chen Y, Zhang L, Hao Q. Candidate microRNA biomarkers in human epithelial ovarian cancer: systematic review profiling studies and experimental validation. *Cancer Cell Int* 2013;13:86.

Colombo PE, Fabbro M, Theillet C, Bibeau F, Rouanet P, Ray-Coquard I. Sensitivity and resistance to treatment in the primary management of epithelial ovarian cancer. *Crit Rev Oncol Hematol* 2013;89(2):207-216.

Drapkin R, von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR, *et al*. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Res* 2005;65:2162-2169.

Eskander RN, Tewari KS. Incorporation of anti-angiogenesis therapy in the management of advanced ovarian carcinoma-mechanistics, review of phase III randomized clinical trials, and regulatory implications. *Gynecol Oncol* 2013;132(2):496-505.

Espada M, Garcia-Flores JR, Jimenez M, Alvarez-Moreno E, De Haro M, Gonzalez-Cortijo L, *et al*. Diffusion-weighted magnetic resonance imaging evaluation of intra-abdominal sites of implants to predict likelihood of suboptimal cytoreductive surgery in patients with ovarian carcinoma. *Eur Radiol* 2013;23:2636-2642.

Fishel ML, He Y, Smith ML, Kelley MR. Manipulation of base excision repair to sensitize ovarian cancer cells to alkylating agent temozolomide. *Clin Cancer Res* 2007;13:260-267.

Fishman DA, Cohen L, Blank SV, Shulman L, Singh D, Bozorgi K, *et al*. The role of ultrasound evaluation in the detection of early-stage epithelial ovarian cancer. *Am J Obstet*

Gynecol 2005;192:1214-1221; discussion 1221-1222.

Gaikwad SM, Ray P. Non-invasive imaging of PI3K/Akt/mTOR signalling in cancer. *Am J Nucl Med Mol Imaging* 2012;2:418-431.

Ganzinelli M, Mariani P, Cattaneo D, Fossati R, Fruscio R, Corso S, *et al*. Expression of DNA repair genes in ovarian cancer samples: biological and clinical considerations. *Eur J Cancer* 2011;47:1086-1094.

Goff BA, Paley PJ, Greer BE, Gown AM. Evaluation of chemoresistance markers in women with epithelial ovarian carcinoma. *Gynecol Oncol* 2001;81:18-24.

Hagmann W, Faissner R, Schnolzer M, Lohr M, Jesnowski R. Membrane drug transporters and chemoresistance in human pancreatic carcinoma. *Cancers* 2010;3:106-125.

Hariri LP, Bonnema GT, Schmidt K, Winkler AM, Korde V, Hatch KD, *et al*. Laparoscopic optical coherence tomography imaging of human ovarian cancer. *Gynecol Oncol* 2009;114:188-194.

Heintz AP, Odicino F, Maisonneuve P, Quinn MA, Benedet JL, Creasman WT, *et al*. Carcinoma of the ovary. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int J Gynaecol Obstet* 2006;95(S1):S161-S192.

Holzer AK, Samimi G, Katano K, Naerdemann W, Lin X, Safaei R, Howell SB. The copper influx transporter human copper transport protein 1 regulates the uptake of cisplatin in human ovarian carcinoma cells. *Mol Pharmacol* 2004;66:817-823.

Holzer AK, Varki NM, Le QT, Gibson MA, Naredi P, Howell SB. Expression of the human copper influx transporter 1 in normal and malignant human tissues. *J Histochem Cytochem* 2006;54:1041-1049.

Howell SB, Safaei R, Larson CA, Sailor, MJ. Copper transporters and the cellular pharmacology of the platinum-containing cancer drugs. *Mol Pharmacol* 2010;77:887-894.

Husseinzadeh N. Status of tumor markers in epithelial ovarian cancer has there been any progress? A review. *Gynecol Oncol* 2011;120:152-157.

Ibanez de Caceres I, Battagli C, Esteller M, Herman JG, Dulaimi E, Edelson MI, *et al*. Tumor cell-specific BRCA1 and RASSF1A hypermethylation in serum, plasma, and peritoneal fluid from ovarian cancer patients. *Cancer Res* 2004;64:6476-6481.

Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, *et al*. MicroRNA signatures in human ovarian cancer. *Cancer Res* 2007;67:8699-8707.

Jordan S, Steer C, DeFazio A, Quinn M, Obermair A, Friedlander M, *et al*. Patterns of chemotherapy treatment for women with invasive epithelial ovarian cancer a population-based study. *Gynecol Oncol* 2013;129:310-317.

Kamazawa S, Kigawa J, Kanamori Y, Itamochi H, Sato S, Iba, T, *et al*. Multidrug resistance gene-1 is a useful predictor of Paclitaxel-based chemotherapy for patients with ovarian cancer. *Gynecol Oncol* 2002;86:171-176.

Katano K, Kondo A, Safaei R, Holzer A, Samimi G, Mishima M, *et al*. Acquisition of resistance to cisplatin is accompanied by changes in the cellular pharmacology of copper. *Cancer Res* 2002;62:6559-6565.

Kinsella TJ. Coordination of DNA mismatch repair and base excision repair processing of chemotherapy and radiation damage for targeting resistant cancers. *Clin Cancer Res* 2009;15:1853-1859.

Krasner CN, Poveda A, Herzog TJ, Vermorken JB, Kaye SB, Nieto A, *et al*. Patient-reported

outcomes in relapsed ovarian cancer: results from a randomized Phase III study of trabectedin with pegylated liposomal doxorubicin (PLD) versus PLD alone. *Gynecol Oncol* 2012;127:161-167.

Kurman RJ, Visvanathan K, Roden R, Wu TC, Shih IeM. Early detection and treatment of ovarian cancer: shifting from early stage to minimal volume of disease based on a new model of carcinogenesis. *Am J Obstet Gynecol* 2008;198:351-356.

Lalwani N, Prasad SR, Vikram R, Shanbhogue AK, Huettner PC, Fasih N. Histologic, molecular, and cytogenetic features of ovarian cancers: implications for diagnosis and treatment. *Radiographics* 2011;31:625-646.

Lee-Jones L. Ovarian tumours: an overview. *Atlas Genet Cytogenet Oncol Haematol* 2004;8:115-119.

Ling KS, Chen GD, Tsai HJ, Lee MS, Wang PH. Mechanisms involved in chemoresistance in ovarian cancer. *Taiwan J Obstet Gynecol* 2005;44(3):209-217.

Martin LP, Hamilton TC, Schilder RJ. Platinum resistance: the role of DNA repair pathways. *Clin Cancer Res* 2008;14:1291-1295.

Matsuzaki H, Kobayashi H, Yagyu T, Wakahara K, Kondo T, Kurita N, *et al*. Plasma bikunin as a favorable prognostic factor in ovarian cancer. *J Clin Oncol* 2005;23:1463-1472.

Menon U, Gentry-Maharaj A, Hallett R, Ryan A, Burnell M, Sharma A, *et al*. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). *Lancet Oncol* 2009;10:327-340.

Motoshima S, Irie H, Nakazono T, Kamura T, Kudo S. Diffusion-weighted MR imaging in gynecologic cancers. *J Gynecol Oncol* 2011;22:275-287.

Musto A, Rampin L, Nanni C, Marzola MC, Fanti S, Rubello D. Present and future of PET and PET/CT in gynaecologic malignancies. *Eur J Radiology* 2011;78:12-20.

Nakayama K, Kanzaki A, Ogawa K, Miyazaki K, Neamati N, Takebayashi Y. Copper-transporting P-type adenosine triphosphatase (ATP7B) as a cisplatin based chemoresistance marker in ovarian carcinoma: comparative analysis with expression of MDR1, MRP1, MRP2, LRP and BCRP. *Int J Cancer* 2002;101:488-495.

Nam EJ, Yun MJ, Oh YT, Kim JW, Kim JH, Kim S, *et al*. Diagnosis and staging of primary ovarian cancer: correlation between PET/CT, Doppler US, and CT or MRI. *Gynecol Oncol* 2010;116:389-394.

Negri E, Franceschi S, Tzonou A, Booth M, La Vecchia C, Parazzini F, *et al*. Pooled analysis of 3 European case-control studies: I. Reproductive factors and risk of epithelial ovarian cancer. *Int J Cancer* 1991;49:50-56.

Nooter K, Herweijer H. Multidrug resistance (mdr) genes in human cancer. *Br J Cancer* 1991;63:663-669.

Olivier RI, Lubsen-Brandsma MA, Verhoef S, van Beurden M. CA125 and transvaginal ultrasound monitoring in high-risk women cannot prevent the diagnosis of advanced ovarian cancer. *Gynecol Oncol* 2006;100:20-26.

Perez RP, Hamilton TC, Ozols RF, Young RC. Mechanisms and modulation of resistance to chemotherapy in ovarian cancer. *Cancer* 1993;71:1571-1580.

Qayyum A, Coakley FV, Westphalen AC, Hricak H, Okuno WT, Powell B. Role of CT and MR imaging in predicting optimal cytoreduction of newly diagnosed primary epithelial ovarian

cancer. *Gynecol Oncol* 2005;96:301-306.

Persidis A. Cancer multidrug resistance. *Nat Biotechnol* 1999;17:94-95.

Rauh-Hain JA, Krivak TC, Del Carmen MG, Olawaiye AB. Ovarian cancer screening and early detection in the general population. *Rev Obstet Gynecol* 2011;4:15-21.

Reichenbach H, Hofle G. Discovery and development of the epothilones : a novel class of antineoplastic drugs. *Drugs R D* 2008;9:1-10.

Richardson A, Kaye SB. Drug resistance in ovarian cancer: the emerging importance of gene transcription and spatio-temporal regulation of resistance. *Drug Resist Update* 2005;8:311-321.

Robinson WR, Barnett G, Rogers AS. Neoadjuvant chemotherapy prior to intraperitoneal chemotherapy in women with advanced ovarian cancer. *Commun Oncol* 2008;5:376-380.

Rose P, Edwards R, Finkler N, Seiden M, Duska L, Krasner C, *et al*. Phase 3 Study: Canfosfamide (C, TLK286) plus carboplatin (P) vs liposomal doxorubicin (D) as 2nd line therapy of platinum (P) resistant ovarian cancer (OC). *J Clin Oncol* 2007;25(18S): LBA5529.

Rutherford T, Orr J, Jr, Grendys E, Jr, Edwards R, Krivak TC, Holloway R, *et al*. A prospective study evaluating the clinical relevance of a chemoresponse assay for treatment of patients with persistent or recurrent ovarian cancer. *Gynecol Oncol* 2013;131:362-367.

Saga Y, Ohwada M, Suzuki M, Konno R, Kigawa J, Ueno S, *et al*. Glutathione peroxidase 3 is a candidate mechanism of anticancer drug resistance of ovarian clear cell adenocarcinoma. *Oncol Rep* 2008;20:1299-1303.

Samimi G, Varki NM, Wilczynski S, Safaei R, Alberts DS, Howell SB. Increase in expression of the copper transporter ATP7A during platinum drug-based treatment is associated with poor survival in ovarian cancer patients. *Clin Cancer Res* 2003;9:5853-5859.

Samimi G, Safaei R, Katano K, Holzer AK, Rochdi M, Tomioka M, *et al*. Increased expression of the copper efflux transporter ATP7A mediates resistance to cisplatin, carboplatin, and oxaliplatin in ovarian cancer cells. *Clin Cancer Res* 2004;10:4661-4669.

Sankaranarayanan R, Ferlay J. Worldwide burden of gynaecological cancer: the size of the problem. *Best Pract Res Clin Obstet Gynaecol* 2006;20:207-225.

Scully RE. Classification of human ovarian tumors. *Environ Health Perspect* 1987;73:15-25.

Selvakumaran M, Pisarcik DA, Bao R, Yeung AT, Hamilton TC. Enhanced cisplatin cytotoxicity by disturbing the nucleotide excision repair pathway in ovarian cancer cell lines. *Cancer Res* 2003;63:1311-1316.

Syng-Ai C, Kumari AL, Khar A. Effect of curcumin on normal and tumor cells: role of glutathione and bcl-2. *Molecular Cancer Therapeut* 2004;3:1101-1108.

Tsujikawa T, Yoshida Y, Mori T, Kurokawa T, Fujibayashi Y, Kotsuji F, *et al*. Uterine tumors: pathophysiologic imaging with 16alpha-[18F]fluoro-17beta-estradiol and 18F fluorodeoxyglucose PET initial experience. *Radiology* 2008;248:599-605.

van Haaften-Day C, Shen Y, Xu F, Yu Y, Berchuck A, Havrilesky LJ, *et al*. OVX1, macrophage-colony stimulating factor, and CA-125-II as tumor markers for epithelial ovarian carcinoma: a critical appraisal. *Cancer* 2001;92:2837-2844.

van Nagell JR, Jr, DePriest PD, Reedy MB, Gallion HH, Ueland FR, Pavlik EJ, *et al*. The efficacy of transvaginal sonographic screening in

asymptomatic women at risk for ovarian cancer. *Gynecol Oncol* 2000;77:350-356.

Vergote I, Finkler NJ, Hall JB, Melnyk O, Edwards RP, Jones M, *et al*. Randomized phase III study of canfosfamide in combination with pegylated liposomal doxorubicin compared with pegylated liposomal doxorubicin alone in platinum-resistant ovarian cancer. *Int J Gynecol Cancer* 2010;20:772-780.

Xiao K, Li Y, Lee JS, Gonik AM, Dong T, Fung G, *et al*. "OA02" peptide facilitates the precise targeting of paclitaxel-loaded micellar nanoparticles to ovarian cancer in vivo. *Cancer Res* 2012;72:2100-2110.

Zhang B, Cai FF, Zhong XY. An overview of biomarkers for the ovarian cancer diagnosis. *Eur J Obstet Gynecol Reprod Biol* 2011;158:119-123.

Zhang Q, Hu G, Yang Q, Dong R, Xie X, Ma D, *et al*. A multiplex methylation-specific PCR assay for the detection of early-stage ovarian cancer using cell-free serum DNA. *Gynecol Oncol* 2013;130:132-139.