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RETRACTED ARTICLE: Nanoscale drug delivery strategies for therapy of ovarian cancer: conventional vs targeted

Swati Gupta, Yashwant Pathak, Manish K. Gupta and Suresh P. Vyas

Amity Institute of Pharmacy, Amity University Uttar Pradesh, Noida, India; College of Pharmacy, University of South Florida Health, Tampa, FL, USA; Faculty of Pharmacy, University of Airlangga, Surabaya, Indonesia; TERI-Deakin Nanobiotechnology Centre, The Energy and Resources Institute (TERI), Gual Pahari, TERI Gram, Gurugram, India; Department of Pharmaceutical Sciences, Dr H.S. Gour University, Sagar, India

ABSTRACT
Ovarian cancer is the second most common gynaecological malignancy. It usually occurs in women older than 50 years, and because 75% of cases are diagnosed at stage III or IV it is associated with poor diagnosis. Despite the chemosensitivity of intraperitoneal chemotherapy, the majority of patients is relapsed and eventually dies. In addition to the challenge of early detection, its treatment presents several challenges like the route of administration, resistance to therapy with recurrence and specific targeting of cancer to reduce cytotoxicity and side effects.

In ovarian cancer therapy, nanocarriers help overcome problems of poor aqueous solubility of chemotherapeutic drugs and enhance their delivery to the tumour sites either by passive or active targeting, and thus reducing adverse side effects to the healthy tissues. Moreover, the bioavailability to the tumour site is increased by the enhanced permeability and retention (EPR) mechanism. The present review aims to describe the current conventional treatment with special reference to passively and actively targeted drug delivery systems (DDSs) towards specific receptors designed against ovarian cancer to overcome the drawbacks of conventional delivery. Conclusively, targeted nanocarriers would optimise the intra-tumour distribution, followed by drug delivery into the intracellular compartment. These features may contribute to greater therapeutic effect.

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Introduction
Ovarian cancer begins when normal cells in an ovary divide and grows uncontrollably, forming a mass called as tumour. A tumour can be benign (noncancerous) or malignant (cancerous, which means it can spread to other parts of the body). The three types of ovarian cancer are epithelial carcinoma, germ cell tumour and stromal tumour. Epithelial carcinoma is the type which shares 85–90% of ovarian cancers. This type of cancer usually begins from cells on the outer surface of the ovary or from fallopian tube epithelium cells [1]. It includes serous tumour, endometrioid tumour and mucinous cystadenocarcinoma.

Epidemiology
Ovarian cancer is considered as the sixth most common cancer in women. It ranks seventh as the cause of cancer death in women worldwide [2]. It accounts for an estimated 239,000 new cases and 152,000 deaths worldwide annually. The highest rates (11.4 per 100,000 and 6.0 per 100,000, respectively) are seen in Eastern and Central Europe [3]. It occurs frequently in white women than African-American women. The United Kingdom has a fairly high incidence rate of 14.6 per 100,000 population. A woman’s risk of getting invasive ovarian cancer in her lifetime is about 1 in 72 and chance of dying from invasive ovarian cancer is about 1 in 100. The incidence rates are highest in Central America and Northern Europe and lowest in some parts of Africa and Asia [4].

American Cancer Society data shows that an estimated 22,440 women would receive a new diagnosis of ovarian cancer and around 14,080 women will die from ovarian cancer in 2017 in the U.S. alone. Ovarian cancer is the fifth most prevalent cancer among women, which accounts for high percentage of deaths when compared to other cancer related to reproductive system cancer. It is stated that 1 in 75 women are at risk of contracting ovarian cancer and fatalities related to the disease is pegged at 1 in 100 [5].

Incidence in India
Ovarian cancer has been reported as one of the most common form of cancer in India. The data reported by Murthy et al. showed that during the period 2001–06, the Age-Standardized incidence Rates (ASR) for ovarian cancer was
0.9 to 8.4 per 100,000 women. Here, Pune and Delhi registries have shown the highest incidence of ovarian cancer. In addition, the trend analysis showed an increasing rate of ovarian cancer incidences with a mean annual percentage increase in ASR in a range of 0.7–2.4%. The Age Specific Incidence Rate (ASIR) for ovarian cancer showed a trend of increasing disease from the age of 35 years and the highest incidences were observed between the ages 55–64 [6].

**Risk factors**

The major factors associated with increase in woman’s risk of developing ovarian cancer are described below [7].

**Age**

Among all age groups, the women of age of 60 and older are at the higher risk of risk of developing ovarian cancer. Data from the Surveillance, Epidemiology, and End Results (SEER) Programme of the National Cancer Institute illustrated the highest occurrence of ovarian cancer in women between the ages of 60 and 85 years. On the basis of age/stage-relationships parameter, the elderly women are more likely than younger women to be in advanced stages of ovarian cancer which constituted about 42% of this group [8].

**Family history**

The women with a family history of ovarian cancer are at the higher risk of developing ovarian cancer than women without such a history. Here, the risk is more for women who have a first-degree relative diagnosed with ovarian cancer. In addition, the chance of developing disease is higher when two or more first-degree relatives have had the ovarian cancer [9].

**Genetics**

BRCA1 and BRCA2 are tumour suppressor genes which play essential roles in DNA repair, cell cycle control, and apoptosis. It is reported that the mutations in both genes increase the risk of developing ovarian cancer [10].

Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC), is a kind of congenital cancer syndrome linked with a genetic predisposition to different cancer types. This means people with Lynch syndrome have a higher risk of certain types of cancer [11]. Women with Lynch syndrome also have an increased risk of developing ovarian cancer.

**Ethnicity**

Women of North American, North European, or Ashkenazi Jewish heritage have an increased risk of ovarian cancer [12].

**Reproductive history**

Women who have never had children or those who had their first child after the age of 30 have an augmented possibility of ovarian cancer. Also, women who started having menstruation before age 12 and/or go through menopause later in life have an increased risk of ovarian cancer [13].

**Hormones**

Women who have used oestrogen-only hormone replacement therapy (HRT) after menopause have a higher risk of ovarian cancer [14].

**Obesity**

Women who were overweight in initial adulthood are 50% more likely to grow ovarian cancer. Obese women are also more likely to die from the disease [14].

**Behavioural and social factors**

Homosexual or bisexual women have a higher risk of ovarian cancer than heterosexual women. Female-to-male transgendered and transsexual people may have a higher risk of ovarian cancer because of receiving hormones [15].

**Clinical presentation**

Early stage cancer is usually asymptomatic [16]. Common symptoms for advanced disease include abdominal swelling, bowel habit changes, vaginal bleeding or pain, urinary frequency or constipation. One of the most important clinical signs in ovarian cancer is a fixed irregular pelvic mass which is best felt by vaginal examination. Pleural effusions, blatant neck nodes and ascites are among the other clinical findings [16,17].

**Staging**

The Federation Internationale de Ginecologie et d’Obstetrique (FIGO) and the American Joint Committee on Cancer (AJCC) have designed staging [18]. Various stages of ovarian cancer are summarised briefly in Figure 1.

**Screening**

Screening of ovarian cancer in individual is complicated due to several limitations including uncertainty in its transition time from stage I to stage III and absence of well-defined precursor lesion. Since relatively less number of ovarian cancer occur in premenopausal women, most screening is suggested for postmenopausal women [19]. Concurrent screening with ultrasonography and CA125 did not diminish ovarian cancer mortality as was observed in a randomised trial in a US population, and evaluation of false-positive results was associated with complications [17,20]. The model tested by Lachance et al. had a sensitivity of 90% and a specificity of 73%, which may further be used for estimating the probability of ovarian cancer [21]. van Nagell et al. observed that a routine sonographic screening not only helps in detection of early stage ovarian cancer in asymptomatic women, but also increases the chances of their 5-year cancer-specific survival [22].
Treatment for ovarian cancer

Surgery

Surgery for ovarian cancer treatment has 2 main goals.

Staging

Usually staging calls for removing the uterus, omentum, both ovaries and both fallopian tubes. Some lymph nodes in the pelvis and abdomen are also taken out. If the abdominal area contains fluid, it will also be removed.

Debulking

The other goal of surgery is to remove as much of the tumour as possible. This is called debulking where the main aim is to leave behind no tumours larger than 1 cm [23].

Radiation therapy

Radiation treatment uses high energy x-rays to kill cancer cells or shrink tumours. The radiation may appear from outside the body or from radioactive materials placed into or near the tumour. Radiation treatment may result into side effects. The skin in the treated area may appear and feel sunburned. Many women also feel fatigue, nausea or diarrhoea [24].

Chemotherapy

Chemotherapy (chemo) is the use of drugs to kill cancer cells or shrink tumours. Most often the drugs are given into a vein (IV) or by mouth [25]. This treatment is particularly advantageous when cancer has spread beyond the ovaries. The drugs can also be given right into the peritoneal cavity. This puts the drugs in contact with the cancer cells while still allowing them to be absorbed to reach the rest of the body. This is termed intraperitoneal (IP) chemotherapy. Various treatment options for ovarian cancer are summarised briefly in Figure 2.

Side effects of chemotherapy

While chemotherapeutic drugs kill cancer cells, they also harm normal cells, causing side effects. Short-term side effects include the following:

- Nausea and vomiting
- Loss of appetite
- Hair loss
- Hand and foot rashes
- Mouth sores

They can damage the cells of the bone marrow resulting in:

- An increased chance of infection (from a shortage of white blood cells)
- Bleeding or staining subsequent to minor cuts (from a deficiency of platelets)
- Tiredness (from low red blood cell counts)

Chemotherapy regimens. Women diagnosed with stage II and higher stage cancer should be advised for front-line chemotherapy treatment. For patients with advanced disease stage, following surgery, taxane or platinum combination (generally carboplatin) and either paclitaxel or docetaxel is prescribed. The normal range of AUC for treatment of ovarian carcinoma varies from 5–8, however, the patients who have received prior chemotherapy or radiation should start with
an AUC of less than 5. Cisplatin at 50–75 mg/m² can be replaced for carboplatin. As compared with standard chemotherapy, increasing the dose intensity of cisplatin did not improve progression-free survival or overall survival [26]. Docetaxel-carboplatin combination has been shown to offer comparable survival rates with less neurotoxicity but greater neutropenia. The combination of paclitaxel and carboplatin is usually given every 3 weeks (day 1 of a 21-d cycle). Katsumata et al. studied the use of a dose-dense regimen, in which paclitaxel was given on day 1, 8, and 15, and carboplatin was given on day [27]. The dose-dense regimen resulted in longer median progression-free survival (28.0 months versus 17.2 months) and higher overall survival at 3 years (72.1% versus 65.1%). Morgan et al. found that an intraperitoneal dose of carboplatin at area under the curve (AUC) of 6 in combination with paclitaxel can be administered with a high rate of completion over multiple cycles [28]. Neutropenia is a recurrent dose-limiting toxicity; thus, haematopoietic growth factors may be added to allow a high completion rate while maintaining this dose. According to a study by Tanner et al., patients receiving adjuvant intraperitoneal chemotherapy are more likely to have recurrences outside the abdominal cavity [29]. Kurtz et al. reported that patients aged 70 years or older are more likely to develop neuropathy and this was even higher in the carboplatin-paclitaxel treated group. The therapeutic index was better among elderly women who received carboplatin-PEGylated liposomal doxorubicin (C AUC 5 plus PLD 30 mg/m² i.v. on day 1 every 4 weeks) than among those who received carboplatin-paclitaxel (C AUC 5 plus P 175 mg/m² i.v. on day 1 every 3 weeks) for six cycles having platinum-sensitive recurrent ovarian cancer [30].

**Intraperitoneal chemotherapy.** Results from clinical trials suggest that intraperitoneal administration of chemotherapy (cisplatin) is superior to intravenous administration in patients with optimally debulked disease [31]. It has been proved that intraperitoneal administration of chemotherapy is associated with an improvement in survival [32,33]. However, this approach is also associated with more toxicity. Jaaback et al. found that intraperitoneal chemotherapy upturns overall survival and progression-free survival in advanced ovarian cancer; but, catheter-related complications and toxicity must also be considered [34].

**Maintenance chemotherapy.** Most ovarian cancer patients achieve a complete clinical response after debulking surgery and platinum-based chemotherapy. However, 50% experience relapse and eventually die of the disease. The experimental chemotherapeutic agent olaparib has shown antitumor activity in patients with high-grade serous ovarian cancer. A phase II study conducted in 2012 evaluated the maintenance treatment with olaparib in patients having platinum-sensitive, relapsed, high-grade serous ovarian cancer. The study showed a progression-free improvement, but no overall survival improvement. Further studies are to be done before olaparib can be recommended in the maintenance chemotherapy [35]. Various chemotherapeutic agents used against ovarian cancer are listed in Table 1.

**Nanotechnology-based approaches in ovarian cancer targeting.**

“Nano” refers to the objects measured in nanometres (nm). Nanotechnology involves the design and engineering of nano objects <500 (nm) in size. Various types of nanoscale drug delivery systems are liposomes, micelles, dendrimers, quantum dots, nanocapsules, nanotubes and nanoparticles (NPs), artificial cells etc. [49,50]. Nanotechnology-based drug delivery systems to the cancer tissue offer better therapeutic properties as they specifically target cancer cells. More bioavailability to the tumour site by the enhanced permeability and retention (EPR) mechanism is obtained. Furthermore, it can also help to overcome the systemic toxicity toward normal cells and cytotoxic effects of conventional chemotherapeutic agents [51]. Therefore, administration of different chemotherapeutic drugs with a suitable nanocarrier could be considered as a promising approach for the treatment of cancer. This targeting can occur in two ways: passive targeting or active targeting. Nanotechnology uses both these modes of targeting to enable specific targeting of cancer cells [52,53]. Figure 3 shows passive and active targeting into tumour cells.

**Passive targeting**

Passive targeting uses the inherent properties of colloidal carriers that lead to the accumulation of nanocarriers at the target site [54]. Targeting cancer cells through passive targeting is easily achieved by using nanocarriers. Passive targeting causes accumulation of nanocarriers in the tumour tissues through the enhanced permeability and retention (EPR) effect [55,56] which leads to higher drug concentrations at tumour sites and thus higher therapeutic efficacy. Various nanocarriers used in ovarian cancer targeting are shown in Figure 4 and described as follows:

**Nanoparticles**

Nanoparticles range from a few nm to several hundred nm depending on their intended use. Because of the deficient tumour lymphatic system, there is EPR of the drug and nanoparticles are accumulated at the tumour site [57].
| Name of drug          | Structure       | Mechanism of action                                      | Side effects                                                                 | Reference |
|-----------------------|-----------------|-----------------------------------------------------------|-------------------------------------------------------------------------------|-----------|
| Cisplatin (Platinol)  | ![Cisplatin](image) | Intrastrand cross-linking of DNA and inhibition of DNA precursors | Nephrotoxicity, nausea, vomiting, changes in hearing, numbness or tingling in hands or feet | 36        |
| Carboplatin (Paraplatin) | ![Carboplatin](image) | Intrastrand cross-linking of DNA and inhibition of DNA precursors | Myelosuppressive effect, neutropenia                                           | 37        |
| Paclitaxel (Taxol)    | ![Paclitaxel](image) | Tubulin polymerisation and microtubule stabilisation.     | Unusual bruising or bleeding, pain/redness/swelling at the injection site, dizziness, shortness of breath, severe exhaustion, skin rash, facial flushing | 38        |
| Doxorubicin (Doxil)   | ![Doxorubicin](image) | Interferes with synthesis of nucleic acid by intercalating with DNA nucleotide pairs and topoisomerase II inhibition. | Nausea, vomiting, heart arrhythmias, typhlitis, discolouration of the urine | 39        |
| Etoposide (Toposar, Etopophos) | ![Etoposide](image) | Single- and double-strand DNA breaks.                     | Low blood pressure, hair loss, pain and or burning at the IV site, metallic food taste, bone marrow suppression | 40        |
| Topotecan (Hycamtin)  | ![Topotecan](image) | Inhibits topoisomerase I, inhibiting DNA replication       | Myelosuppression, diarrhoea, low blood counts, susceptibility to infection     | 41        |
| Name of drug          | Structure                                                                 | Mechanism of action                                                                                   | Side effects                                                                                       | Reference |
|-----------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|-----------|
| Gemcitabine (Gemzar)  | ![Gemcitabine Structure](image)                                             | Inhibits ribonucleotide reductase and competes with deoxycytidine triphosphate for incorporation into DNA | Lower resistance to infection, anaemia, flu-like illness, skin changes                              | 42        |
| Docetaxel (Taxotere)  | ![Docetaxel Structure](image)                                              | Promotes assembly and blocks the disassembly of microtubules                                          | Alopecia, neutropenia, anaemia, febrile neutropenia and thrombocytopenia                           | 43        |
| Vinorelbine (Navelbine) | ![Vinorelbine Structure](image)                                            | Inhibits tubulin polymerisation during G2 phase of cell division                                      | Lower resistance to infection, bruising or bleeding, anaemia, nausea, numbness or tingling in hands or feet, inflammation of the vein. | 44        |
| Ifosfamide            | ![Ifosfamide Structure](image)                                             | DNA cross-linking and denaturation of double helix                                                   | Encephalopathy (brain dysfunction), normal anion gap acidosis, specifically renal tubular acidosis type 2 | 45        |
| Fluorouracil (Adrucil)| ![Fluorouracil Structure](image)                                           | Disrupts DNA synthesis or cellular viability                                                        | Sore mouth and ulcers, taste changes, gritty eyes, blurred vision.                                | 46        |
| Melphalan (Alkeran)   | ![Melphalan Structure](image)                                              | Inhibits mitosis by cross-linking DNA strands                                                        | Nausea and vomiting, oral ulceration, bone marrow suppression, pulmonary fibrosis, hair loss, interstitial pneumonitis, rash, itching. | 47        |
| Altretamine (Hexalen) | ![Altretamine Structure](image)                                             | Inhibits DNA and RNA synthesis by inhibiting the incorporation of radioactive thymidine and uridine into DNA and RNA | Nausea, vomiting, anaemia and peripheral sensory neuropathy                                        | 48        |
Figure 3. Passive and active targeting of nanoparticles in tumour cells. Passive targeting refers to accumulation of nanocarriers in the tumour tissues due to their inherent properties through the enhanced permeability and retention (EPR) effect. Passive targeting ensues as a result of the distinctive pathophysiological features of tumour vessels. Actually, the diffusion constraint encountered by tumour growth of above a size of around 2 mm results into hypoxia and enhanced angiogenesis. The subsequent disparity of angiogenic regulators such as growth factors and matrix metalloproteinase creates immature tumour vasculature: new vessels are extremely disordered and expanded, and the endothelium becomes leaky. Additionally, lymphatic drainage is poor. Accretion of macromolecules or nanocarriers that escape out of the fenestrated vasculature is jointly termed the enhanced permeability and retention effect. Active targeting utilises the principle of ligand–receptor recognition to deliver the drug carrier/cargo directly to the cells which exhibit a specific receptor and which may facilitate drug uptake through numerous phagocytosis and endocytosis mechanisms. To stimulate such receptors, a distinguishable cancer-specific ligand must be anchored onto the carrier surface.

Figure 4. Various nanocarriers used in ovarian cancer targeting. Nanotechnology has a substantial effect on therapeutic effectiveness of drugs. These nanocarriers are capable of substituting the conventional available chemotherapeutic treatments, where in the intravenous injection of most of cytotoxic agents results in serious dose-limiting side effects to healthy tissues. Drug resistance, associated with the delivery of antineoplastic agents can be minimised or reduced by applying the concept of nanotechnology together with combination therapy.
Cisplatin-loaded nanoparticles having pH-sensitive poly (2-((N, N-diethylamino) ethyl methacrylate) (PDEA) cores were synthesised from PDEA-block-poly (ethylene glycol) (PDEA-PEG) copolymer using a solvent displacement method. The PDEA-PEG nanoparticles dissolved at pH <6, thereby rapidly released cisplatin into cytoplasm upon integration into acidic lysosomes and thus overcame the chemoresistant activity of SKOV-3 cells. Cellular pyknosis (irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis) was most apparent within intestinal tumours of mice treated with cisplatin/PDEA-PEG and the tumour growth was also reduced [58]. A novel acid-responsive poly (ethylene glycol)-block-poly (l-lactide) (PEG-PLA) polymer-cisplatin pro-drug conjugate nanoparticle was reported. The nanoparticles displayed increased in vitro activity against ovarian tumour cells as compared to free cisplatin. Also, these nanoparticles can decrease the drug loss in systemic circulation where pH value is neutral and cause rapid intracellular drug release when the nanoparticles endocytosed by the target cells. This acid-responsive drug release kinetics may effectively suppress cancer cell proliferation and enhance the therapeutic efficacy of the anticancer drug [59]. In a study by Feng et al., the efficacy of poly-(γ-L-glutamylglutamine)-paclitaxel (PGG–PTX) was compared to that of Abraxane, which is an established nanoparticulate formulation of paclitaxel (PTX). PGG–PTX produced considerably greater ovarian tumour inhibition than Abraxane. Thus, PGG–PTX has the potential to surpass Abraxane in increasing the delivery of PTX to tumours while at the same time reducing the toxicity of both single dose and weekly treatment regimens [60]. Work done by Cafaggi et al. showed that nanoparticles with the smallest size and the lowest positive zeta potential induced apoptosis in A2780 human ovarian carcinoma cells. Mean particle diameter varied from 180 nm to 350 nm. These results show that cisplatin complexes with polycarboxylate polymers can be of high potential interest [61]. Paclitaxel-loaded poly-(3-hydroxybutyric acid-co-hydroxyvaleric acid) (PHBV) nanoparticles were prepared by Vilos et al. The size range of nanoparticles was found to be 228–264 nm which made them suitable for passive targeting by EPR effect. These showed a low in vitro rate of release, intracellular uptake and a high cytotoxicity capacity in cell lines and in primary cultures of stage IIIc serous ovarian cancer cells. These results and the low cost to synthesise PHBV make it a potential candidate for the development of novel drug-loaded nanoparticles for large-scale pharmaceutical production [62].

**Solid-lipid nanoparticles (SLN)**

SLN are spherical particles made from solid lipids with a mean diameter between approximately 50 and 1000 nm. They can be derived from the emulsions for parenteral administration by replacing the liquid lipid (oil) of the emulsion droplets by a solid lipid [63]. SLN are advantageous as compared to polymeric nanoparticles as they can be produced by high pressure homogenisation identical to parenteral O/W emulsions [64]. To develop an alternative formulation of paclitaxel suitable for parenteral administration, sterically stabilised solid lipid nanoparticles loaded with paclitaxel were prepared by Lee et al. Treatment of the OVCAR-3 cell line and the MCF-7 breast cancer cell line with paclitaxel-loaded SLNs yielded cytotoxicities which were similar to those of a commercially available Cremophor EL-based paclitaxel formulation. These results recommend that SLN based formulation might have a potential as an alternative delivery system for parenteral administration of paclitaxel [65].

**Liposomes**

Liposomes are closed spherical vesicles consisting of a lipid bilayer that encapsulates an aqueous core. The liposomal diameter varies from 400 nm to 2.5 mm. The liposomal bilayer can be composed of either synthetic or natural phospholipids. The process of liposome formation is natural because the amphiphilic phospholipids join together to form bilayers. Liposomes generally reach their site of action by extravasation into the interstitial space from the bloodstream. Liposomes can be used for both active and passive targeting [66]. Liposomes are PEGylated to reduce clearance by the MPS (mononuclear phagocytic system), thereby increasing the circulation half-life [67]. PEGylated cisplatin loaded liposomes with diameters of about 110 nm and targetability to transferrin receptors (TfR) were prepared to compare cisplatin cell uptake with cytotoxicity in sensitive and cisplatin resistant ovarian cancer cells A2780. Although, TfR targeting had less impact on activity in comparison to non-targeted liposomes. Overall, this study strongly supports approaches to overcome cisplatin resistance by using a liposomal formulation of the drug [68]. In another study, the Mn2+ gradient coupled with A23187 ionophore was applied in the sequential co-encapsulation of doxorubicin and irinotecan, into the liposomes. The co-encapsulated liposome formulation was further tested in an intraperitoneally grown, human ovarian tumour xenograft model, and was shown to significantly improve the survival of the tumour-bearing animals [69].

In a recent study, Qi et al. evaluated the efficacy of pegylated liposomal formulation containing paclitaxel (PL-PTX) in inhibiting the growth of ovarian cancer cells in *in vitro* and *in vivo* models. The PL-PTX treatment significantly suppressed the growth and aggressiveness of ovarian cancer cells. In addition, PL-PTX induced the ovarian cancer cell apoptosis via TNF induced ERK/AKT signalling pathway. It is also reported that the PL-PTX treatment increases the expression of caspase 3/9, ERK and AKT in ovarian cancer cells [70].

**Micelles**

Micelles are spherical or globular structures that are formed when the hydrophobic end of molecules cluster to form the central core of the sphere. The hydrophilic ends of the molecules are thus in contact with the liquid environment surrounding the micelle and form a covering. The hydrophobic central core carries the water insoluble drugs [71]. The doxorubicin (DOX)-loaded polymeric micelles exhibited a potent cytotoxicity against human A2780 ovarian carcinoma cells. These results show that novel polymer micelles with cross-linked ionic cores can be suitable carriers for the delivery of
DOX [72]. Poly (ethylene glycol)-block-poly (ε-caprolactone) (PEG-b-PCL) micelles loaded with paclitaxel (PTX) (cytotoxic agent), cyclopamine (CYP) (hedgehog inhibitor), and gossypol (GSP) (Bcl-2 inhibitor) were prepared. PEG-b-PCL micelles carrying PTX, CYP, and GSP were biocompatible, physically stable in aqueous solution and capable of simultaneous sustained release of PTX, CYP, and GSP in vitro. This 3-drug combination was highly efficient in metastatic ES-2-luc and SKOV3-luc xenograft models. These results prove that PEG-b-PCL micelles have a potential to be used in ovarian cancer treatment based on drug combinations of cytotoxic agents and molecular targeted agents, delivered concomitantly [73].

Parthenolide (PTL), a hydrophobic anticancer drug-loaded micelle nanoparticles based on reproducible self-assembly of diblocks, poly(styrene-alt-maleic anhydride)-b-poly(styrene) (PSMA-b-PS) and poly(styrene-alt-maleic anhydride)-b-poly(ethyl acrylate) (PSMA-b-PBA) were synthesised. The hydrophobic PSMA-b-PS micelles (PSMA100-b-PS258) which showed highest PTL loading and prolonged release profile were further loaded with doxorubicin (DOX), as well as nile red and IR-780 (hydrophobic fluorescent probes). The study showed that PTL is released quantitatively within 24 h, while the release of DOX, IR-780, and nile red last over a week. It is suggested that the slow release of hydrophobic molecules from the nanoparticles having hydrophobic core is attributed to the favorable drug-core interactions and weaker drug-solvent interactions. DOX-loaded PSMA-b-PS micelles exhibited greater cytotoxicity to multidrug resistant ovarian cancer cells (NCI/ADR RES cells) compared with equivalent free DOX doses [74].

**Nanocapsules**

Nanocapsules are vesicular systems with a central cavity or core to which a drug is bound. The core is surrounded by an outer shell polymeric membrane to which surface bound targeting ligands or antibodies may be attached [75]. At a dose of 3 mg cisplatin/kg, cisplatin nanocapsules and cisplatin in solution exhibited similar therapeutic efficacy, reducing tumour growth by 90% at day 20 after first injection. The study concludes that the cisplatin nanocapsules formulation inhibits the growth of OVCAR-3 xenografts in nude mice, even though a similar extent as free cisplatin [76]. In a multidrug-resistant ovarian cancer cell line, OVCAR-3, lapatinib/PTX nanocolloids showed an improved tumour inhibition in comparison with the PTX-only treatment [77].

**Dendrimers**

These are regularly branched, three-dimensional, treelike structures with a central core molecule. Branch lengths have steric limitations so the dendrimer formed are spherical shaped with small molecular size but high molecular weights. Drug molecules may either be attached to functional groups on the dendrimer surface or secured in the interior environment of the sphere [78,79]. These can host both hydrophobic and hydrophilic carrier molecules. In a study, the free dendrimers showed no cytotoxicity while the cisplatin–dendrimer complexes showed moderate activity. Cisplatin, at its maximum tolerated dose of 6 mg/kg, reduced tumour size by 33% compared to an untreated control group. The G6.5 cisplatin–dendrimer complex was administered at two doses (6 and 8 mg/kg equivalent of cisplatin). The lower dose displayed a tumour volume reduction of 32%, but the higher dose was significantly more active with a tumour reduction of 45% than free cisplatin [80]. Also, Cai et al. developed a linear-dendritic copolymer system (referred to as telodendrimer) via peptide chemistry for the co-delivery of paclitaxel, a low dose hydrophobic drug, and cisplatin, a hydrophilic drug. The single-drug loaded telodendrimers displayed less cytotoxicity than free drugs due to the slow release profile; however, telodendrimers loaded with two drugs showed significantly higher cytotoxicity against SKOV3 cells, indicating a strong synergism. Optimal cytotoxicity was observed at a 2:1 ratio of cisplatin:paclitaxel. In in-vivo fluorescence optical imaging studies, fluorescent dye encapsulated telodendrimers accumulated mainly at the SKOV-3 tumour xenograft, 4-fold higher than other organs; whereas free dye showed very weak tumour fluorescence [81].

**Hydrogel**

Hydrogels are three-dimensional structures consisting of cross-linked networks of water-soluble polymers. Hydrogels also possess a degree of flexibility due to their considerable water content [82]. A depot formulation is created from which drug is slowly released, thus maintaining a high local concentration of drug in the surrounding tissues over an extended period [83]. One of the major challenges in IP chemotherapy is the need to provide effective drug concentrations in the peritoneal cavity for an extended period of time. To overcome this, hyaluronic acid (HA)-based in-situ crosslinkable hydrogel was prepared for the regional delivery of paclitaxel (PTX) to the IP tumours. PTX was best held in the peritoneal cavity as PTX-gel (microparticulate PTX entrapped in the HA gel) followed by PTX-suspension (microparticulate PTX particles). Taxol-based formulations (Taxol-gel, Taxol, and Taxol-multiple) were cleared from the peritoneal cavity and were not detected after 14 days. Due to the limited dissolution of PTX, PTX-gel and PTX-suspension did not further increase the therapeutic effects against the IP tumours. Thus, HA gel may enhance the therapeutic effect of a drug if paired with particles of an optimum size that provide continuous and unimpeded supply of PTX [84]. In another study, an in-situ crosslinkable hydrogel depot containing paclitaxel (PTX) nanocrystals (PNC) was developed to facilitate IP chemotherapy of ovarian cancer. PNC suppressed SKOV3 cell proliferation more efficiently than microparticulate PTX precipitates (PPT), and the gel containing PNC (PNC-gel) showed a lower maximum tolerated dose than PPT-containing gel (PPT-gel) in mice, indicating greater dissolution and cellular uptake of PNC than PPT. A single IP administration of PNC-gel extended the survival of tumour-bearing mice significantly better than Taxol, but PPT-gel did not [85].
Polymer-drug conjugates
Polymer-drug conjugates are drug molecules held up in polymer molecules. Polymer drug conjugates are preferably accumulated in solid tumours and can reduce systemic toxicity and so are useful in cancer diagnosis and treatment. Polymers are used as carriers for the delivery of proteins, drugs, targeting moieties and imaging agents [86]. Several polymers, poly (ethylene glycol) (PEG), N-(2-hydroxypropyl) methacyrlamide (HPMA) and poly (lactide-co-glycolide) (PLGA) copolymers have been successfully utilised in clinical research [87]. In this study, two polymer-drug delivery systems (P-HYD1-DOX and P-HYD2-DOX) were developed by conjugating doxorubicin (DOX) to a poly (L-lysine citramide) (PLCA) polymer carrier with a hydrazone-based degradable spacer. P-HYD-DOX conjugates showed a significant activity against ovarian tumour and their ability to release free and active DOX in i.p. deposits and tumour. DOX was conjugated to the PLCA carrier by a cleavable spacer arm based on the hydrolysis-sensitive hydrazone chemical function. These can further be studied for i.p. chemotherapy after cytoreductive surgery in the management of ovarian cancer [88]. The above-mentioned studies are summarised in Table 2.

Active targeting
Active targeting utilises the principle of ligand–receptor recognition to deliver the chemotherapeutic agent directly to the cells which exhibit a specific receptor [89]. Cancer cells may express some specific receptors on their surface that are absent from normal cells, or some receptors on cancer cells may be overexpressed. Active targeting involves a nanocarrier with a specific ligand that has a high affinity to a tumour cell differentiating target receptor. Some examples of tumour differentiating target receptors are folate receptor alpha (FRα), EGFR, HER2 receptor, CA125 receptor, and TAG-72 [90,91]. Various receptors for active targeting of ovarian cancer are depicted in Figure 5.

Targeting folate receptor
The folic acid receptor is considered as useful target for tumour specific drug delivery, specifically in distortions of the ovary. The folate receptor is overexpressed in numerous cancers, predominantly in ovarian carcinoma [93] as folic acid is an indispensable vitamin consumed enormously by proliferating cells for the biosynthesis of nucleotide bases [92]. Furthermore, the density of folate receptor upsurges as the grade of the cancer exacerbates. As polarity is lost in cancer cells, folate receptors become easily available to targeted drugs delivered in the plasma [1].

Folate receptor (FRα)-mediated targeting of gold nanoparticles (AuNPs) to cancer cells have been studied. Among ovarian cancer cells, the expression pattern of FRs followed the order OV-167 > SKOV-3 > OV-202 > OVCAR-5, and for multiple myeloma cell lines the order was OPM-1 > U266 > RPMI. Maximum uptake is observed for OV-167, whereas it is minimum for OVCAR-5 [94]. Attenuated measles viruses are promising experimental anticancer agents for ovarian cancer patients. A single-chain antibody (scFv) specific for α-folate receptor (FRα), was genetically engineered on the viral attachment protein (MV-αFR). A FR-exclusive ovarian cancer-targeted oncolytic virus was generated and shown to be therapeutically effective, thus introducing a new carrier for FR targeting [95]. Zhang et al. prepared bovine serum albumin nanoparticles (BSANPs) by coacervation method and chemical cross-linking with glutaraldehyde. The association of folate-conjugated BSANPs to SKOV3 cells was inhibited by an excess amount of folic acid which suggested that the binding and/or uptake were mediated by the folate receptor. These results showed that the folate-conjugated BSANPs might be useful as a drug carrier system to deliver drugs into the cells over expressing folate receptor [96]. In the study conducted by Wang et al., significant uptake of the folic acid-conjugated polyglycerol-grafted Fe3O4@SiO2 (FA-HPG-grafted Fe3O4@SiO2) nanoparticles by human ovarian carcinoma cells (SKOV-3) as compared to macrophages and fibroblasts was observed. These nanoparticles can potentially be used to provide real-time imaging in ovarian cancer resection [97]. Nanogels are comprised of swollen polymer networks and contain nearly 95% water and can entrap different chemical and biological agents for cancer therapy with high loading capacities. Diblock copolymer poly (ethylene oxide)-b-poly (methacrylic acid) (PEO-b-PMA) was used to form nanogels with the desired degree of cross-linking. The nanogels were further conjugated to folic acid (FA) and loaded with different types of drugs (cisplatin, doxorubicin). This proves the potential of nanogels for ovarian cancer therapy [98]. ChemoRad NP is the one which can deliver both chemotherapy and radiotherapy. A folate-targeted ChemoRad NP encapsulating paclitaxel (Ptxl) and yttrium-90 (Y90) for IP chemoradiotherapy of ovarian cancer was prepared. Paclitaxel was used as the chemotherapeutic drug and Y90 as the therapeutic radioisotope. An in vitro study showed that the folate targeted-NP Ptxl Y90 was a more effective chemoradiotherapy than non-targeted NP Ptxl Y90. In vivo study showed that folate-targeted NP Ptxl Y90 is more effective than folate-targeted-NP Ptxl, folate-targeted-NP Y90, non-targeted NPs containing Ptxl or Y90, and free Ptxl. Thus, a ChemoRad NP could be of potential use in the treatment for ovarian tumour [99]. Turk et al. studied the distribution of folate-targeted liposomes. According to flow cytometric analysis, folate-conjugation of liposomes considerably increased their uptake into ovarian cancer cells and tumour-associated macrophages within tumour ascites fluid. Macrophages acquired tenfold more liposomes as compared to ovarian cancer cells. This study thus showed that folate-liposomes may be useful for targeting drugs to cancer cell and to tumour-associated macrophages in vivo. It can be concluded that removal of both of these cell populations would be favourable for the treatment of ovarian cancer [100]. In another study, the micelleplexes formed upon electrostatic interaction with siRNA are used to deliver siRNA in a targeted manner to SKOV-3 ovarian cancer cells that overexpress folate receptor-α (FRα). Furthermore, toll-like receptor 4 (TLR4) knock down within SKOV-3 cells resulted from siRNA delivery resensitized them toward paclitaxel (PTX) treatment, and apoptotic events increased [101].
Nevertheless, Ganta et al. developed a folate targeted gadolinium decorated theranostic nanoemulsion of docetaxel for overcoming efflux transporters which are one of the chemoresistance mechanisms of ovarian cancer and tracing drug distribution. According to cellular uptake studies, theranostic nanoemulsion uptake into folate receptor positive SKOV3 ovarian cancer cell line was time dependent and higher than nontargeted nanoparticles. MTT studies demonstrated that IC50 value of chemoresistant SKOV3TR decreased 270-fold compared to free drug. Magnetic resonance imaging study showed that folate targeted theranostic nanoparticles accumulated over the period of 24 h at tumour site [102]. Li et al. developed tumour-targeted siRNA/folic acid–poly(ethylene glycol)–chitosan oligosaccharide lactate

Table 2. Various nanocarriers for passive targeting to ovarian cancer.

| Carrier system | Drug encapsulated | Description | Cell lines used | Animal model used | Reference |
|---------------|-------------------|-------------|-----------------|-------------------|-----------|
| Nanoparticles | Cisplatin         | Copolymer of pH responsive (PDEA) poly[2-(N,N- diethylaminol)ethyl methacrylate] - block-PEG (poly ethylene glycol) (PDEA-b-PEG) | SKOV-3 | Female athymic (nu/nu) mice (BALB/c strain) | 58 |
|               | Cisplatin         | Poly(ethylene glycol)-b-poly(l-lactide) (PEG-PLA) diblock copolymer conjugated through an acid liable bond | A2780 | — | 59 |
|               | Paclitaxel (PTX)  | Poly-(γ-L-glutamylglutamine)–poly-γ-L-glutamylglutamine (PGG-PTX) nanoparticle | 2008 | Female athymic nude (nu/nu) mice | 60 |
| Polymeric nanocarriers | Cisplatin | Chitosan or N-trimethyl chitosan nanoparticle non-covalently bound to cisplatin-alginate complex | A2780 | — | 61 |
|               | Paclitaxel | Poly(3-hydroxybutyric acid-co-hydroxyserine acid) (HPB) nanoparticles (NP-Taxel) | Ishikawa, SKOV3 | — | 62 |
| Lipid-based nanocarriers | Paclitaxel | Solid lipid nanoparticle with trimyristin core and egg phosphatidylcholine and PEGylated phospholipid as stabilisers | OVCAR-3 | — | 65 |
|               | Cisplatin | Liposomes based on Soy phosphatidylcholine, cholesterol, polyethylene glycol phosphatidylethanolamine (SPC/Chol/ mPEG-PE) | A2780, A2780cis | — | 68 |
| Doxorubicin and irinotecan | Doxorubicin | Pegylated liposomes | CAOV-3 | — | 69 |
| Polymers micelle | Doxorubicin | Polymeric micelle of poly(ethylene oxide)-block-poly(methacrylic acid) (PEG-b-PMA) | CAOV-3 | — | 70 |
|               | Paclitaxel | Poly(ethylen glycol)-block-poly(-caprolactone) micelle | ES-2, SKOV3 | Female athymic nude-Foxn1nu mice | 73 |
| Parthenolide & doxorubicin | Micelle nanoparticles based on diblocks, poly(styrene-alt-maleic anhydride-b-polystyrene) (PSMA-b-PS) and poly(styrene-alt-maleic anhydride-b-poly(butyl acrylate) (PSMA-b-PBA) | multidrug resistant (NCI/ADR RES cells) | — | — | 74 |
| Nanocapsule | Cisplatin | Composed of 1, 2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), 1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethylene glycol)2000] (DOPE-PEG2000) and cholesterol | OVCAR-3 | Female nude mice (Hsd: athymic nude-nu) | 76 |
|               | Paclitaxel (PTX) and lapatinib | PTX/chitosan/alginate acid core-shell structure. PTX/chitosan/alginate acid core-shell structure. | OVCAR-3 | — | 77 |
| Dendrimer | Cisplatin | Anionic half generation 3.5–6.5 poly(amidoamine) dendrimers | A2780, A2780cis, A2780cp | Female athymic nude mice | 80 |
|               | Paclitaxel and cisplatin | Linear-dendritic copolymer system (referred to as telodendrimer) | SKOV3 and ES-2 | Female SPF BALB/c mice for pharmacokinetic studies and Nude mice bearing human SKOV3 ovarian cancer tumour for antitumor efficacy | 81 |
| Hydrogel | Paclitaxel | Hyaluronic acid (HA)-based in-situ crosslinkable hydrogel | SKOV-3 | Female athymic (nu/nu) mice | 84 |
|               | Paclitaxel (PTX) nanocrystals (PNC) | PNC containing Hyaluronic acid (HA) gel | SKOV-3 | Female Balb/c nude mouse | 85 |
| Polymer-drug conjugate | Doxorubicin | Doxorubicin (DOX) conjugated to a poly(L-lysine citramide) polymer carrier with a hydrazone-based degradable spacer. | SKOV-3 | Female, BALB/c mice | 88 |
(FA–PEG–COL) nanoparticles for hypoxia inducible factor (HIF)-1a suppression and therefore inhibition of angiogenesis and tumour growth. In vitro gene silencing studies made via Western Blot and Real time PCR demonstrated that siRNA delivery by FA–PEG–COL nanoparticles significantly reduced both protein and mRNA levels of HIF-1a, leading to a strong suppression of cell proliferation in human ovarian cancer cells. When fluorescent dye loaded FA–PEG–COL nanoparticles iv administered to nude mice bearing OVK18#2 human ovarian cancer cells, significant accumulation at the tumour site was seen at 3 h post injection with subsequent increase at the 12 and 24 h time while significant liver accumulation was observed with COL nanoparticles treated animal group, indicating the active targeting ability of FA–PEG–COL nanoparticles [103].

Guliz et al. [104] developed doxorubicin loaded, glucose/gluconic acid-coated and folate receptor-targeted magnetically responsive nanoparticles for ovarian cancer therapy. In folate-free medium, folate receptor-targeted nanocarrier showed 10.33-fold lower IC50 values for A2780 cells and 3.93-fold lower for OVCAR3 cells compared to non-targeted nanoparticles and demonstrated more cytotoxicity against ovarian cancer cells. Moreover, magnetically and folate receptor-targeted doxorubicin delivery system was significantly more effective for therapy of xenografted nude mice than free doxorubicin based on tumour shrinkages and biochemical parameters.

Mo et al. [105] designed a novel folic acid-PEG-conjugated p-phosphonated calix[4]arene nanoparticle (Fp-PCN) for the simultaneous delivery of paclitaxel (PAC) and carboplatin (CAR) at an optimal ratio (5:1, mol:mol) to utilise their potential synergistic effect against OC cells. The Fp-PCNs loaded with PAC and CAR (Fp-PCNPAC + CAR) resulted in a remarkable efficacy in the suppression of OC, both in vitro and in vivo. Compared to free drugs, Fp-PCNPAC + CAR showed stronger apoptosis induction as well as invasion and self-renewal capacity suppression in SKOV-3 cells. The molecular mechanism to address the synergism is that Fp-PCNPAC + CAR down regulated JMJD3 expression to modulate the H3K27me3 epigenetic mark of the promoters of HER2 and MYCN. Furthermore, the expressions of JMJD3 and HER2 were significantly associated with poor outcomes for ovarian patients. The study demonstrates that co-delivery of PAC and CAR can be achieved with the Fp-PCNs, and reveals a previously unrecognised and unexpected role of the JMJD3-HER2 signalling axis in PAC and CAR treatment of OC.

Paclitaxel (PTX)-loaded nanoparticles (NPs) (PTX-PEG-PLA-NP and PTX-PEG-PLA-FA-NP) were prepared by Yao et al. A drug-distribution study of tumour-bearing mice demonstrated that the PTX concentration of tumour tissues in the PTX-PEG-PLA-FA-NP group was three times higher than the other two groups. PTX-PEG-PLA-FA-NP was uptaken much more by SK-OV-3 cells than PTX-PEG-PLA-NP and free PTX. The tumour inhibitory rate in the PTX-PEG-PLA-FA-NP group of tumour-bearing mice was found to be about 1.5 times higher than the controls thus shows potential to be a new therapy strategy for ovarian cancer patients [106].
**Targeting luteinizing hormone-releasing hormone (LHRH) receptor**

LHRH is a decapetide that binds to receptors on pituitary gonadotropes, stimulating biosynthesis and secretion of follicle stimulating hormone (FSH) and LH, which regulate gonadal steroidogenesis and gametogenesis in both sexes. LHRH receptor represents a useful target in sex hormone-dependent tumours as it is expressed in human breast (52%), ovarian (80%), endometrial (80%), and prostate (86%) carcinomas. The antitumor effects of cecropin B, an antimicrobial peptide coupled LHRH’ (a form of LHRH modified at carboxyl-terminal residues 4–10) i.e. CB-LHRH’ was evaluated in three drug resistant ovarian cancer cell lines (SKOV-3, ES-2, NIH:OVCAR-3) and an endometrial cancer cell line (HEC-1A). CB-LHRH’ significantly inhibited the cell viability of SKOV-3, ES-2, NIH:OVCAR-3 and HEC-1A, but not that of normal eukaryotic cells. Also, CB-LHRH’ inhibited tumour growth with a 23.8 and 20.4% reduction in tumour weight at 50 and 25 mg/kg.d, respectively [107].

Functionalization with LHRH peptide via phospholipid, the face centred cubic (fcc) iron-platinum (FePt) NPs can bind preferentially to the human ovarian cancer cell line (A2780) that over-expresses LHRH receptors, and showed high toxicity to these tumour cells. The Fe released catalysed H2O2 decomposition into reactive oxygen species within cells, thus, causing fast oxidation and deterioration of cellular membrane [108]. Also, targeted nanogel was prepared for selective delivery of cisplatin to LHRH receptor overexpressing tumour in vivo. LHRH-nanogel accumulation was found to be specific to the LHRH-receptor positive A2780 ovarian cancer cells and not toward LHRH-receptor negative SKOV-3 cells. The LHRH-nanogel cisplatin formulation was more effective and less toxic than equimolar doses of free cisplatin or untargeted nanogels in the treatment of receptor-positive ovarian cancer xenografts in mice [109].

Dharap et al. [110] synthesised three different conjugates of Camptothecin and polyethylene glycol utilising luteinizing hormone-releasing hormone (LHRH) and BCL-2 homology 3 (BH3) peptide as a targeting moiety and a suppressor of cellular anti-apoptotic defence, respectively (CPT-PEG, CPT-PBH3 and CPT-PHELHRH) and examined in A2780 human ovarian cancer cells. Cytotoxicity, expression of genes encoding BCL-2, BCL-XL, SMAC, APAF-1 proteins and caspases 3 and 9, the activity of caspases 3 and 9 and apoptosis induction were studied and the results indicated much higher cytotoxicity and apoptosis-inducing activity of PEG-CPT conjugates when compared to free CPT. Moreover, the effects of targeted CPT-PBH3 and CPT-PHELHRH conjugates were more pronounced than the non-targeted PEG-CPT conjugate.

**Targeting HER2 receptors**

The HER2 receptors having tyrosine kinase activity are a member of the receptors of epidermal growth factors (EGF) family. These receptors are overexpressed in various types of human cancers specifically in breast cancer, but also in epithelial ovarian cancer. Tumour cell propagation and survival pathways are the result of HER2 intensification and subsequent HER2 protein overexpression, therefore, drugs have been developed to target this pathway [111,112].

Paclitaxel (Tx)-loaded nanoparticles coated with anti-HER2 monoclonal antibodies (Herceptin®, trastuzumab) were assessed in a disseminated xenograft ovarian cancer model induced by intraperitoneal inoculation of SKOV-3 cells overexpressing HER2 antigens. A considerably longer survival of mice was observed for NPs–Tx–HER treatment compared to free Tx, Tx-loaded nanoparticles coated with an irrelevant mAb (Mabthera®, rituximab) or Herceptin® alone, indicating the probable use of immuno-nanoparticles in ovarian cancer treatment. Thus, proving that NPs–Tx–HER immuno-nanoparticles resulted in an improved efficacy of drug in the treatment of disseminated ovarian cancer over expressing HER2 receptors [113].

**Targeting FSHR receptor**

The FSH, the follicle-stimulating hormone binds to ovarian receptor, FSHR. FSHR is a G-protein coupled receptor with seven-transmembrane domains. Ovarian surface epithelium articulates this receptor which is restricted to the reproductive system. The manifestation of this receptor is upheld and even overexpressed on ovarian cancer cells. The progression of cancer cells by increasing the cells propagation is supported by the existence of this receptor [114].

A peptide derived from FSH (amino acids 33–53 of the FSH β chain, named as FSH33), was used to develop a conjugated nanoparticle, FSH33-NP, to target FSHR (Follicle-stimulating hormone receptor) in ovarian cancer. FSH33-NP-PTX showed higher antiproliferation and antitumor effects when compared with free PTX or naked PTX-loaded nanoparticles (NP-PTX) both in vitro and in vivo [115] In another study, receptor ligand-based anti-FSHR immunoreceptors were constructed that contained small binding fragments from the ligand for FSHR, FSH, fused to T-cell transmembrane and T-cell signalling domains. Human T cells transduced to express anti-FSHR immunoreceptors were specifically immunoreactive against FSHR-expressing human and mouse ovarian cancer cell lines and mediated effective lysis of FSHR-expressing tumour cells, but not FSHR-deficient targets, in vitro. Similarly, the outgrowth of human ovarian cancer xenografts in immunodeficient mice was significantly inhibited by the adoptive transfer of FSHR-redireced T cells [116].

Furthermore, the targeted poly(amidoamine) (PAMAM) dendrimers conjugated with the binding peptide domain of FSH (FSH33) exhibited high receptor selectivity to FSHR-expressing OVCAR-3 cells as compared to SKOV-3 cells that do not express FSHR. Immunostaining of the conjugates revealed their selective binding and uptake by ovarian surface epithelium (OSE) cells that express FSHR while sparing the immature primordial follicles. Also, in vivo study showed significantly higher accumulation of FSH33-targeted dendrimers in the ovary and oviduct compared to the non-targeted conjugates [117].

Fan et al. [118] developed a paclitaxel loaded nanoparticle system targeting the follicle stimulating hormone receptor (FSHR) to prevent lymphatic metastasis of ovarian cancer. Targeted nanoparticles showed improved cellular uptake into
FSHR positive cells, NuTu-19, while there was no difference for non-targeted nanoparticles between FSHR positive and negative cells. In a model of ovarian cancer with lymphatic metastasis in rats, the drug concentration in lymph nodes for the animal group treated with targeted nanoparticles was observed to increase over time and was significantly higher than the free drug and non-targeted nanoparticle group. Moreover, the size and weight of the lymph nodes were reduced and average survival time was longer for the targeted nanoparticle group [118].

Targeting transferrin receptors (TfR)
The TfR is a cell membrane-associated glycoprotein involved in the cellular uptake of iron and in the regulation of cell growth. TfR is overexpressed in many solid tumours, ovarian cancer cells in particular (A2780, OVCA429, OVCA432, OVCAR-3, SKOV3 and HEY). Transferrin or the antibodies against the transferrin receptor (for instance, R17217 and OX26 monoclonal antibody) can be used to target TfR. In a study, transferrin (Tf)-modified poly(ethylene glycol)-phosphatidylethanolamine (mPEG-PE) micelles loaded with the poorly water-soluble drug, R547 (a potent and selective ATP-competitive cyclin-dependent kinase (CDK) inhibitor), were prepared and evaluated for their targeting efficiency and cytotoxicity in vitro and in vivo to A2780 ovarian carcinoma cells, which overexpress TfR. Tf-modified micelles showed enhanced interaction with A2780 ovarian carcinoma cells in vitro. The in vitro cytotoxicity and in vivo tumour growth inhibition studies in A2780-tumour bearing mice confirmed the enhanced efficacy of Tf-modified R547-loaded micelles compared to free drug solution and to nonmodified micelles [119].

Targeting integrin receptors
Integrins are transmembrane receptors that expedite binding between cell and extracellular matrix (ECM). Upon ligand binding, integrins trigger signal transduction pathways that arbitrate cellular signals such as controlling of the cell cycle, organisation of the intracellular cytoskeleton, and movement of new receptors to the cell membrane. Kulhari et al. [120] designed polymeric nanoparticles for targeted intracellular delivery of gemcitabine hydrochloride (GEM) utilising the unique ability of a cyclic pentapeptide cRGDfK to specifically target αvβ3 integrin receptors that are overexpressed on SKOV-3 human ovarian cancer cells. This significantly increased the effective intracellular drug concentration even at low doses, thereby remarkably improving the chemotherapeutic potential of GEM. cRGDfK-conjugated, GEM-loaded nanoparticles reduced the non-specific haemolytic cytotoxicity of the drug, simultaneously influencing intracellular processes such as mitochondrial membrane potential (ΔΨm), reactive oxygen species (ROS) levels, and apoptosis, thereby favourably influencing drug antiproliferative efficacy.

Targeting ovarian cancer biomarkers
Tumour markers are antigen or protein secreted by the tumour itself or the adjacent tissues in response to the tumour and can be identified in the serum of patients. Preferably, tumour markers must be both precise and sensitive for the malignancy and signal recurrence before clinical symptoms occur. CA-125 is a large antigen (250 kDa), lacking in normal ovarian tissues, but articulated in more than 80% cases in ovarian cancer patients. CA-125 is the most common serum biomarker used for detection of ovarian cancer, and its sensitivity is associated with the clinical stage of the disease. It can also be used to specifically target ovarian cancer cells due to its expression at the surface of the ovarian cancer cells also [121].

A single-chain antibody variable domain (scFv) that recognises the CA125 antigen of ovarian cancer cells was fused with a core-streptavidin domain using recombinant DNA technology and then expressed in Escherichia coli using the T7 expression system. The confocal laser scanning microscopy (CLSM) study showed specificity in binding to the OVCAR-3 cell line. ELISA and western blot studies confirmed the bifunctional activity and specificity. In the presence of bfFp (bifunctional fusion protein), there was better binding of biotinylated antigen and liposome to OVCAR-3 cells. Whereas, the control cells, which do not express the CA125 antigen, showed minimal binding of the bfFp. Thus, bfFp targeting of biotinylated therapeutic drugs, liposomes or nanoparticles could be an alternative and suitable method to deliver effective therapy for ovarian cancer patients [122].

For delivery of liposomes containing proteins or nucleic acids, it would be beneficial to avoid endocytosis to prevent degradation in the lysosomes. The pairing of HIV-1 derived TAT-peptide to the outer surface of liposomes increases their binding to ovarian carcinoma cells. It was revealed that cellular uptake of TAT-liposomes occurs via endocytosis rather than plasma membrane translocation. Thus, extent of endocytic uptake is improved using TAT-peptides covalently coupled to liposomes, which might be advantageous [123].

Ovarian cancer cells were targeted by coupling paclitaxel (Tx)-loaded nanoparticles (NPs-Tx) to antibodies against KDEL sequence, which is able to recognise GRP94 and GRP78 that are located at cell surface in cancer cells whereas they are found in the endoplasmic reticulum in healthy cells. GRP78 plays a critical role in the tumour neovascularization during tumour growth and metastasis, linkage of cell surface with antibodies against its C-terminal domain may contribute to inhibit neovascularization. It thus confirmed that coating Tx-loaded nanoparticles with anti-KDEL antibodies was able to bind to Bg-1 cells and enhanced the cytotoxicity of the drug in vitro [124].

Doxorubicin was encapsulated in polyethylene glycol-1-phosphatidyl ethanolamine (PEG-PE) conjugated micelles. The doxorubicin loaded PEG–PE micelles (MDOX) were then incubated with a cancer cell-specific monoclonal 2C5 antibody to obtain doxorubicin-loaded immunomicelles (2C5-MDOX). Accumulation and toxicity of doxorubicin-loaded PEG–PE micelles were evaluated in three dimensional ovarian cancer cell spheroid model. Higher accumulation of 2C5-MDOX compared to free doxorubicin or untargeted MDOX in spheroids was observed. As spheroids are detected in peritoneal cavities of ovarian cancer patients, intraperitoneal administration of 2C5-MDOX could be a better option against...
primary and distant ovarian carcinoma. On the whole, these results support the use of spheroids to evaluate targeted drug delivery against cancer [125].

Biodegradable NPs of poly(lactic-co-glycolic acid) (PLGA) loaded with shikonin (a cytotoxic agent with immunomodulatory effects) were formulated. The surface of NPs was further decorated with solubilising agent polyethylene glycol (PEG) and tumour endothelial marker 1 (TEM1)/endosialin-targeting antibody (Ab). Fluorescence microscopy and flow cytometry analysis showed active interaction of Ab-armed NPs with TEM1-positive M51 cells, but not with TEM1-negative M51 cells. While exposure of the PE Gylated NPs for 2 h was not toxic to lymphocytes, long-term exposure of the Ab-armed and PEGylated NPs was significantly toxic to TEM1-positive M51 cells and OVCAR-5 cells [126].

Furthermore, doxorubicin-loaded lyophilisomes (albumin-based biocapsules) were functionalised to specifically target the stroma of ovarian carcinomas with the potential to eliminate cancer cells. Antibody-functionalised lyophilisomes specifically targeted the ovarian cancer stroma through highly sulphated chondroitin sulphate, CS-E. In a CS-E rich microenvironment in vitro lyophilisomes induced cell death by extracellular release of doxorubicin which localised to the nucleus [127].

Targeting angiogenesis

Angiogenesis (the formation of new blood vessels) is responsible for tumour growth and survival as it delivers the nutrients and the oxygen essential to maintain tumour cell biological functions. Zheng et al. [128] developed silica nanoparticle (SLN) for the tumour-targeted co-delivery of two anti-angiogenic drugs, candesartan (CD) and trastuzumab (Tra), for ovarian cancer therapy via different anti-angiogenic mechanisms using hyaluronic acid (HA)/Tra/CD/SLNs. In vitro and in vivo anti-angiogenic assays showed that CD and Tra suppressed cancer angiogenesis, and exhibited significantly enhanced effects compared with the angiotensin stimulated group (p < .01). CD and Tra co-delivery also significantly increased the anti-angiogenic effect compared with applying either drug alone (p < .01). Furthermore, HA anchoring reduced the cytotoxicity and enhanced the tumour-homing property in vitro and in vivo of developed SLN.

Magnetic nanoparticles

Magnetic nanoparticles generally comprise of two constituents, a magnetic material, frequently iron, nickel and cobalt, and a chemical component/drug that has functionality/therapeutic activity work under magnetic fields. An antitumor drug delivery system based on carboplatin prodrug loading Fe3O4 nanoparticles (NPs@carboplatin) was developed and the antitumor activity was also investigated. It exhibited a higher cytotoxic effect than carboplatin on both A2780 (cisplatin sensitive) and A2780DDP (cisplatin resistant) ovarian cancer cells via MTT assay, which can overcome Pt resistance. Moreover, the nanoparticles (NPs) loaded with carboplatin possess excellent delivery capability, which can be effectively taken up by ovarian cancer cell lines through an endocytosis process. Furthermore, in vivo experiments demonstrated that NPs@carboplatin can be widely distributed into major organs, and in the presence of an external magnetic field, the Fe3O4 nanocarrier is beneficial to visualise the tumour site location and promote the subsequent antitumor efficacy [129].

Miscellaneous

Paclitaxel (PTX)-loaded cationic nanostructured lipid nanoparticles (PTX-NLCs) were prepared. Hyaluronic acid (HA)-PE was then coated onto the PTX-NLCs by electrostatic adsorption to form HA-PTX-NLCs. SKOV3 human ovarian cancer cells (SKOV3 cells) and PTX-resistant SKOV3 cells (SKOV3/PTX cells) were used to analyse in vitro tumour cell inhibition efficiency. In vivo antitumor ability was evaluated with mice bearing SKOV3 ovarian cancer cells xenografts. The in vitro viability of SKOV3 cells and SKOV3/PTX cells was noticeably inhibited by HA-PTX-NLCs. In the ovarian cancer cells model, significant decrease in tumour growth was perceived, whereas the conventional PTX injection group did not attain significance. These findings deeply support the supremacy of HA-based nano-system for the PTX delivery, thus augment the effectiveness of ovarian cancer chemotherapy [130].

In a study, Rh2–treated graphene oxide (GO-Rh2), lysinetreated highly porous graphene (Gr-Lys), arginine-treated Gr (Gr-Arg), Rh2–treated Gr-Lys (Gr-Lys-Rh2) and Rh2–treated Gr-Arg (Gr-Arg-Rh2) were synthesised. MTT assay was used for evaluation of cytotoxicity of samples on ovarian cancer (OVCAR3). Interestingly, Gr-Arg, Gr-Lys, Gr-Arg-Rh2, and Gr-Lys-Rh2 were more active against cancer cell lines in comparison with their cytotoxic activity against normal cell lines (MSCs) with IC50 values higher than 100 µg/ml. The results of TUNEL assay indicated a significant increase in the rates of TUNEL positive cells by increasing the concentrations of nanomaterials [131].

Table 3 summarises the various types of active carrier systems along with their targeted receptor.

Plant-derived agents against ovarian cancer

Since ancient times, plants have been a main source of various conventional drugs for the treatment of different types of cancer. Although the actual plant products may not serve as drugs, they actually provide leads for the development of novel anti-cancer agents. The search for anti-cancer agents from plant sources started in the 1950s. A recent analysis verified that 62% of anti-infective and antitumor agents either commercially available or in late stages of development are drugs of natural origin. Plant-derived compounds include vinca alkaloids like vinblastine and vincristine, camptothecin derivatives, topotecan and irinotecan, etoposide and paclitaxel (taxol®). Plants produce a variety of chemicals such as terpenes, phenols, alkaloids, steroids and organosulfates which show unique antiproliferative effects at a subtoxic level. Many new potential agents are in clinical trials based on their activity as anti-cancer agents, while some agents which failed earlier in clinical trials are invigorating new interest [132].
| Targeted receptor/antigen | Carrier system | Description | Tumour cell lines used | Animal model used | Ref. |
|--------------------------|----------------|-------------|-----------------------|-------------------|-----|
| Folate receptor α (FRα)  | Gold nanoparticle | Gold nanoparticle (AuNP) conjugated to PEG with folic acid noncovalently bond to the AuNP | SKOV-3, OVCAR-5, OV-202, OV-167 | — | 94 |
| Viral nanoparticle       | Measles virus genetically engineered with the viral attachment protein expressing a single-chain antibody specific for FRα | SKOV-3, IGROV-1 | Severe combined immunodeficient mice— for *in vivo* targeting experiments, measles susceptible Ifnar-CD46Ge transgenic mice— for virus biodistribution analysis, female athymic mice for the therapy experiments | 95 |
| Albumin nanoparticle     | Bovine albumin nanoparticle conjugated with folic acid | SKOV-3 | Female nude athymic (nu/nu) mice | 96 |
| Magnetic nanoparticle    | Folic acid-PEG-conjugated Fe₃O₄@SiO₂ (FA-PEG-grafted Fe₃O₄@SiO₂) nanoparticles | SKOV-3 | Female nude athymic (nu/nu) mice | 97 |
| Nanogel                 | Diblock copolymer poly(ethylene oxide)-block-poly(methacrylic acid) (PEO-b-PMAA) | A2780 | Female nude athymic (nu/nu) mice | 98 |
| ChemoRad nanoparticle   | ChemoRad NP encapsulating paclitaxel (Ptxl) and yttrium-90(Y90) | SKOV-3 | Female nude athymic (nu/nu) mice | 99 |
| Liposomes               | Composed of egg phosphatidylcholine, cholesterol and polyethylene glycol— derivatized phosphatidylethanolamine (PEG2000-PE) | IGROV | Female athymic mice | 100 |
| Micelleplexes           | Micelleplexes formed upon electrostatic interaction with siRNA to deliver siRNA for paclitaxel treatment | SKOV-3 | — | 101 |
| Nanoemulsion            | Gadolinium decorated theranostic nanoemulsion of doxorubicin | SKOV3 | Female nu/nu mice | 102 |
| Nanoparticles           | Folic acid– polyethylene glycol– chitosan oligosaccharide lactate (FA–PEG–COL) nanoparticles for delivery of siRNA | OV1882 | Nude mice bearing OV1882 human ovarian cancer cells | 103 |
| Nanoparticles           | Glucose/glucosamine coated, doxorubicin loaded magnetically responsive nanoparticles | A2780 and OVCAR3 cells | Xenografted nude mice | 104 |
| Nanoparticles           | Folic acid-PEG-conjugated p-phosphonated calix[4]arene nanoparticle (Ff-PCN) for the simultaneous delivery of paclitaxel (PAC) and carboplatin (CAR) | SKOV-3 cells | nu/nu female BALB/c mice | 105 |
| Nanoparticles           | Pegylated folic acid-conjugated Paclitaxel loaded Polyacrylic acid nanoparticles | SKOV-3 cells | Female athymic mice (BALB/c nu/nu) | 106 |
| Peptide conjugate       | Cecropin B, an antimicrobial peptide coupled LHRH (a form of LHRH modified at carboxyl-terminal residues 4–10) | ovarian cancer cell lines (SKOV-3, ES-2, NIH-OVCAR-3) and an endometrial cancer cell line (HEC-1A) | Female athymic nude mice (BALB/c-nu) | 107 |
| Magnetic nanoparticle   | Iron-platinum or iron oxide nanoparticle with PEGylated phospholipid (PL) and PL conjugated to luteinizing hormone-releasing hormone peptide | A2780 | — | 108 |
| Nanogel                 | Poly(ethylene glycol)170-b-poly(methacrylic acid)180 (PEG-b-PMAA) diblock copolymer based cisplatin nanogel | A2780 and SKOV-3 | Female athymic nu/nu mice | 109 |
| PEG-Drug conjugate      | Conjugates of camptothecin and polyethylene glycol utilising luteinizing hormone-releasing hormone (LHRH) and BCL-2 homology 3 (BHS) peptide as a targeting moiety | A2780 human ovarian cancer cells | — | 110 |
| HER2 receptor           | Poly(DL-lactic acid) nanoparticle loaded with paclitaxel and conjugated to anti-HER2 (trastuzumab, Herceptin) | SKOV-3, SKOV3-Iuc-D3 | Females BALB/cOlaHsd mice and C.B-17/ScidMice | 113 |
| Follicle Stimulating Hormone (FSH) receptor | Poly(DL-lactic acid) nanoparticle loaded with paclitaxel and conjugated to anti-HER2 (trastuzumab, Herceptin) | Ca0v-3, ES-2, OVCAR-3, SKOV-3 | Female BALB/c mice | 115 |

(continued)
| Targeted receptor/antigen | Carrier system | Description | Tumour cell lines used | Animal model used | Ref. |
|--------------------------|---------------|-------------|------------------------|-------------------|------|
| Receptor ligand-based anti-FSHR immunoreceptors | Dendrimers | Poly(amideamine) (PAMAM) dendrimers conjugated with the binding peptide domain of FSH (FSH33) | OVCAR-3 & SKOV-3 | Female BALB/c mice | 117 |
| Nanoparticle | FSH peptide [81–95 (QCHCGKCDSTDCT)] modified PEG-PLA paclitaxel loaded NPs | NuTu-19 and SKOV-3 | Female rat with lymphatic metastasis | 118 |
| Transferrin receptors (TfR) | Micelles | Transferrin (Tf)-modified poly(ethylene glycol)-phosphatidylethanolamine (mPEG-PE) micelles loaded with the poorly water-soluble drug, R547 (a potent and selective ATP-competitive cyclin-dependent kinase (CDK) inhibitor) | A2780 | Female nu/nu mice | 119 |
| Integulin receptors | Nanoparticle | Cyclic pentapeptide cRGDFK conjugated polymeric nanoparticles containing gemcitabine-hydrochloride (GEM) | SKOV-3 | — | 120 |
| Ovarian cancer biomarkers | Liposomes towards CA125 antigen | Bifunctional fusion protein composed of a variable chain of anti-CA125 fused to streptavidin which binds to biotinylated liposomes | OVCAR-3 | — | 122 |
| Liposomes conjugated with TAT-peptide | Prepared using egg-phosphatidylcholine (EPC), cholesterol, Maleimide-PEG2000-DSPE and 1,2-distearoyl-glycerol-3-phosphoethanolamine-N-poly(ethylene glycol)2000(PEG2000-DSPE) | OVCAR-3 | — | 123 |
| Nanoparticles towards KDEL sequence | Tx-loaded poly (DL-lactic acid) nanoparticles coated with anti-KDEL antibodies (NPs-Tx-KDEL) | Bg-1 | — | 124 |
| Monoclonal 2C5 antibody conjugated Molecule | Doxorubicin encapsulated in polyethylene glycol-phosphatidyl ethanolamine (PEG–PE) conjugated micelles | NCI-ADR-RES | — | 125 |
| Nanoparticles | PEG and Ab conjugated poly(lactic-co-glycolic acid) (PLGA) NPs loaded with shikonin (a cytotoxic agent with immunomodulatory effects) | MS1 cells and OVCAR-5 cells | — | 126 |
| Lyophilosomes | Antibody-functionalised doxorubicin loaded albumin-based biocapsules to target highly sulphated chondroitin sulphate (CS-E) present in the ovarian cancer extracellular matrix | CS-E producing (SKOV3 and SKOV3F7) and non-CS-E (HFF1 cells; human foreskin fibroblasts) | — | 127 |
| Angiogenesis | Nanoparticle | Silica nanoparticles for co-delivery of two anti-angiogenic drugs, candesartan (CD) and trastuzumab (Tr) | SKOV-3 | Female BALB/c nude mice | 128 |
| Magnetic Nanoparticles | Carboblatin prodrug loaded Fe3O4 nanoparticles | A2780 (cisplatin sensitive) and A2780DDP (cisplatin resistant) ovarian cancer cells | KM mice H22 xenograft tumour model | 129 |
The aqueous extract of herb, *Herba Scutellaria barbatae* (HSB), induced apoptosis in proliferating but not growth-arrested ovarian cancer cell lines. Apoptosis was measured by nuclear and DNA fragmentation and Annexin V binding. The aqueous extract of HSB displayed excellent anticancer activity against 11 ovarian cancer cell lines and 2 breast cancer cell lines tested [133].

The effects of *Duchesnea* phenolic fraction (DPF) on SKOV-3 ovarian cancer cells were studied. DPF showed cytotoxicity towards human ovarian cancer SKOV-3 cells through induction of apoptosis. It induced apoptosis via mitochondrial pathway and arresting cell cycle progression in S phase. DPF suppressed Bcl-2 levels, improved Bax levels and Bax/Bcl-2 ratio, and at the same time translocated Bax to mitochondria followed by mitochondrial release of cytochrome c into the cytosol and activation of effector caspase-3. Besides, DPF provoked S phase arrest in SKOV-3 cells with down-regulation of cyclin A, E, D1 and CDK2 [134].

Ginkgo extract and its components, quercetin and ginkgolide A and B, had significant anti-proliferative effects (~40%) in serous ovarian cancer cells, but little effect in mucinous (RMUG-L) cells. The inhibitory effect for ginkgolides was due to cell cycle blockage at G0/G1 to S phase [135].

The in vitro anti-proliferation activities of Devil’s club *Oplopanax horridus* (OH) root bark extracts, on cisplatin sensitive and resistant human ovarian cancer cell lines, were studied. Water, 70% ethanol, 100% ethanol, and ethyl acetate extracts of OH inhibited the proliferation of human ovarian cancer cell lines A278, A2780CP70, OVCAR3 and OVCAR10 in vitro. Combinations of 70% ethanol OH extract with cisplatin and paclitaxel increased its anti-proliferation effect on A2780 and A2780CP70 cells. At low concentrations, it induced cell death by apoptosis, while at high concentrations, it killed cells by necrosis [136].

Antiproliferation activity of rosemary extract (RE) and its three main active ingredients carnosol (CS), carnosic acid (CA) and rosmarinic acid (RA) were studied against human ovarian cancer cells. The results showed that RE had major antiproliferation activity on human ovarian cancer A2780 and its cisplatin resistant daughter cell line A2780CP70. A2780 cells were more responsive to CS, CA and RA than A2780CP70 cells between 2.5 and 20 μg/ml. CS and RA also showed synergistic antiproliferation effect with cisplatin on A2780 cells. This study showed that RE holds potential to be used in ovarian cancer chemotherapy [137].

The crude methanolic extract of *P. daemia* is a prospective source of natural antioxidants and thus it could be used in cancer therapy. The reducing power assay, ABTS assay and FRAP assay showed that the plant extract had a good antioxidant property [138].

Curcumin significantly inhibited the growth and induced apoptosis in Ho-8910 cells. A decrease in Bcl-2, Bcl-XL, and pro-caspase-3 expression was observed after exposure to 40 μM curcumin while the levels of p53 and Bax were increased in the curcumin-treated cells. This data might contribute to the anticarcinogenic action of curcumin [139]. Cisplatin resistant A2780CP ovarian cancer cells were pre-treated with curcumin followed by exposure to cisplatin or radiation. The poly (lactic acid-co-glycolic acid) (PLGA) nanoparticle formulation of curcumin (Nano-CUR) was developed by a modified nano-precipitation method. Pre-treatment with curcumin significantly reduced the dose of cisplatin and radiation required to inhibit the growth of cisplatin resistant ovarian cancer cells. Nano-CUR had an average particle size of ~70 nm, showed steady and prolonged release of curcumin and inhibition of ovarian cancer cell growth. Thus, PLGA nanoparticle formulation of curcumin might improve the in vivo therapeutic efficacy of curcumin [140].

Hypericin (Hy), a natural photosensitizer (PS) extracted from *Hypericum perforatum*, had been efficient in vitro and in vivo for the treatment of other cancers, so it could also be a potent means for the treatment and detection of ovarian cancer. Polymeric nanoparticles (NPs) of polylactic acid (PLA) or polylactic-co-glycolic acid (PLGA) were used as drug delivery system. Their in vitro photoactivity was examined on the NuTu-19 ovarian cancer cell model derived from Fischer 344 rats and compared to free drug. Hy-loaded PLA NPs showed a higher photoactivity than free drug. Increasing the light dose or incubation time with cells increased activity of Hy-loaded PLA NPs [141].

CC chemokine receptor 5 (CCR5) and its ligand CC chemokine ligand 5 (CCL5/RANTES) are significant in proliferation and invasion of ovarian cancer. Anibamine, which is CCR5 antagonist and its analogues were studied for their effects on proliferation of the OVCA3 ovarian cancer cells. The compounds also inhibited the proliferation of OVCAR-3 at micromolar to submicromolar range. Based on these results, anibamine and one of its synthetic analogues seemed as potential leads to develop novel anti-ovarian cancer agents [142].

Zhang et al. reported that tetrandrine significantly increased the cytotoxicity of cisplatin in ovarian cancer. The in vitro assay showed that tetrandrine could increase apoptosis induced by cisplatin. Further assay indicated that modulation of Wnt/cadherin signalling pathway contributed to the chemosensitizing effect of tetrandrine on the cytotoxicity of cisplatin in ovarian cancer. In vivo studies were done on female athymic nude mice (BALB/c, nu/nu). These results proved that the combination of tetrandrine and cisplatin could be used in ovarian cancer therapy [143].

The effects of Withaferin A were studied in CaOV3 and SKOV3 ovarian carcinoma cell lines by MTS assay, clonogenic assay, annexin V/protopidium iodide flow cytometry, and cell cycle analysis. Withaferin A-induced apoptosis and inhibited colony formation of CaOV3 and SKOV3 cells. These changes were correlated with Notch1, Notch3, cdc25C, total and phosphorylated Akt and Bcl-2 proteins down-regulation which are critically involved in ovarian cancer progression [144].

Phenolic extract (APEI) showed higher antiproliferative activity in drug-sensitive and drug-resistant ovarian cancer cell lines by inducing rapid accumulation of cells in sub-G1 phase. Also, it had selective activity in ovarian cancer cell lines compared with non-tumorigenic ovarian cells. Thus, it has potency to be used in the treatment of patients with ovarian cancer [145].

Kenaf (*Hibiscus cannabinus*) is a fibre plant which is rich source of biologically active compounds in the class of...
Phenolic fraction of dextrins, micelles and nanogels. In ovarian cancer therapy, conjugates, dendrimers, inclusion complexes based on cyclo-ovarian cancer are nanoparticles, liposomes, polymer-drug systems which are reconnoitred majorly in the targeted therapy of specific into malignant ovarian cells. The drug carriers systems which are capable of carrying the therapeutic agents substantial efforts employed to engineer various nano carrier sys-tems which are tested as single agents, therefore, several immune processes may need to be manipulated simultaneously using nanocarriers help overcome problems of poor aqueous solu-bility of chemotherapeutic drugs and enhance targeting either by passive or active manner, thus reducing adverse side effects of chemotherapeutic drugs. Presently, numerous strategies are used to target receptors either present naturally or overexpressed at the surface of ovarian cancer cells, or due to existence of biomarkers. The results of preclinical studies executed on these different nano systems indicate active targeted strategy as favourable treatment of ovarian cancers. Additional benefit of using these nanocarriers is drug release at a minimum effective concentration over a period of time. Active targeting strategy is associated with an enhanced drug accumulation at solid-tumour sites. Various studies show that active targeting could be of added significa-cance to ovarian anticancer therapy. Thus, nanotechnology and various plant extracts are presenting new opportunities for improving ovarian cancer therapy.

Nowadays, immune therapy targeting the immune check-point receptors such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death 1 (PD-1), and pro-grammed cell-death ligand 1 (PD-L1) are among the most favourable approaches, having proven clinical activity in a wide variety of tumours. To date, immune checkpoint inhibitors tested as single agents, therefore, several immune processes may need to be manipulated simultaneously using combinatorial approaches of conventional therapy, novel target-ed agents and immunotherapy. It is projected that future biomarker-directed clinical trials will provide further percep-tions into the mechanisms underlying response and resistance to immunotherapy, and assist in designing therapeutic combinations having potential to improve the benefit of immunotherapy in patients with ovarian cancer.

Table 4 Various extracts prepared from natural plants showing activity against ovarian cancer.

| Plant extracts and their source                      | Cell lines used                      | Reference |
|------------------------------------------------------|--------------------------------------|-----------|
| Aqueous extract of herb Herba Scutellaria barbatae   | A2780, SKOV3, CAOV3, OVCAR-3, Hey, HeyC2, HA8, OCC | 133       |
| Phenolic fraction of Duchesnea indica (Andr) Focke   | SKOV-3                               | 134       |
| Crude extract of Ginkgo biloba                      | OVC4A29, OVC4A33, OVC4A20, HOSE-6E67, RMUG-5, RMUG-L | 135       |
| Root bark extracts of Devil’s club (Oplopanax hederifolius) | A2780, A2780CP70, OVCAR3, and OVCAR10 | 136       |
| Rosemary extract (RE) obtained from Rosmarinus officinalis L | A2780, A2780CP70 | 137       |
| Crude methanolic extract of the plant Pergularia daemia | OAW-42, PA-1                          | 138       |
| Curcumin (diferuloyl methane), a polyphenol extracted from the rhi zomes of tumeric, Curcuma longa | Ho-8910                                 | 139       |
| Hypericin (Hy), a natural photosensitizer (PS) extracted from Hypericum perforatum | A2780, A2780CP                        | 140       |
| Anibamine, a pyridine quaternary alkaloid isolated from Aniba panurensis | OVCAR-3                                | 142       |
| Tetrandrine (Tet), a bisbenzylisoquinoline alkaloid, extracted from the root of the creeper Stephania Tetrandrinandra | NIH-OVCAR-3, A2780                    | 143       |
| Withaferin A (WA), a steroidal lactone extracted from Withania somnifera | CaOV3, SKOV3, OVCAR3, TOV112D, TOV21G    | 144       |
| Phenolic extract (APE1) from a native Chilean plant of the Amaranthaceae family. | A2780, HEY, OVCAR8, HOSE2            | 145       |
| Kenaf seed oils extracted from Kenaf (Hibiscus cannabinus) belonging to family Malvaceae | CaOV3                                 | 146       |
| Honokiol, phenolic constituent of magnolia bark (Magnolia officinalis) | SKOV3, COC1, A2780                      | 147       |

tannins, saponins, polyphenolics, alkaloids, essential oils and steroids. Yazan et al. reported the cytotoxicity of kenaf seed oil on ovarian cancer (CaOV3) and colon cancer (HT29) cell lines. The kenaf seed oil was found to be the most effective against CaOV3 cell line. The oils treated cells showed chromatin condensation and nuclear fragmentation, suggesting that the cytotoxic activity of kenaf seed oil towards the investigated cancer cells may be attributed to the apoptosis induced cell death [146].

Honokiol inhibited cell proliferation and induced apoptosis in ovarian tumour cells by alteration of Bcl-2 members and caspase-3. It also controlled tumour growth and inhibited angiogenesis in vivo, with no evidence of cytotoxicity. These results demonstrated that honokiol might be a potential candidate for ovarian cancer treatment [147]. Table 4 enlist the plant products that are useful in ovarian cancer treatment.

**Conclusions and future directions**

Ovarian cancer is the leading cause of death from gynecological malignancies worldwide. Optimal management of ovarian cancer requires a multidisciplinary approach that includes appropriate preoperative imaging, skilful surgical staging and debulking, careful histopathologic diagnosis and optimally delivered chemotherapy. A more aggressive debulking surgery and the IP administration of chemotherapy might provide some survival benefit but toxicities and poor practicability of these treatments severely limit their potential efficacy. However, combinations of chemotherapeutic agents show significant activity with acceptable toxicity in patients with ovarian cancer, whether used as first-line therapy or in the salvage setting. The precise dosing and different mechanisms of action of combinatorial drugs make them attractive for these combinations. Recent literature demonstrates substantial efforts employed to engineer various nano carrier systems which are capable of carrying the therapeutic agents specifically into malignant ovarian cells. The drug carriers which are reconnoitred majorly in the targeted therapy of ovarian cancer are nanoparticles, liposomes, polymer-drug conjugates, dendrimers, inclusion complexes based on cyclo-dextrins, micelles and nanogels. In ovarian cancer therapy, nanocarriers help overcome problems of poor aqueous solubility of chemotherapeutic drugs and enhance targeting either by passive or active manner, thus reducing adverse side effects of chemotherapeutic drugs. Presently, numerous strategies are used to target receptors either present naturally or overexpressed at the surface of ovarian cancer cells, or due to existence of biomarkers. The results of preclinical studies executed on these different nano systems indicate active targeted strategy as favourable treatment of ovarian cancers. Additional benefit of using these nanocarriers is drug release at a minimum effective concentration over a period of time. Active targeting strategy is associated with an enhanced drug accumulation at solid-tumour sites. Various studies show that active targeting could be of added significance to ovarian anticancer therapy. Thus, nanotechnology and various plant extracts are presenting new opportunities for improving ovarian cancer therapy.

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