



Targeted Agents in Ovarian Carcinoma

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Abstract

Gynecologic malignancies take an important part in female cancers. Ovarian cancer is the second most common gynecologic cancer with the highest mortality rate in developed countries. Most of the patients need an adjuvant chemotherapy regimen after the initial surgery. Despite their suppressive effect on tumor cells, much toxicity on healthy cells could be seen with these standard chemotherapeutics. In that manner a new way of tumor cell disruption with less toxicity and cancer selective effect is needed, which is called targeted therapy. Moreover this approach could prevent chemo-resistance and increase chemo-sensitivity of the disease. Primary cytoreductive surgery with platinum based chemotherapy shape the initial management of these tumors. However, recently we are on the edge of molecular therapy for this cancer. In this setting we need to identify a dominant biological pathway for tumor progression and discover a functional and structural molecule within this pathway. The new therapeutic modalities with targeted molecules should build up new perspectives insight tumor cell behaviour. Agents against angiogenesis and receptors of growth factors in addition to signalling cascades and intracellular enzymes could shape the individual treatment protocols for cancer survivors.

Keywords: Targeted therapy, ovarian carcinoma, chemotherapy

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Targeted Agents Overview

Gynecologic malignancies take an important part in female cancers that uterine corpus and ovarian cancer are in the ten leading cancer types for both new cases and deaths. While endometrium cancer is the most common gynecologic malignancy, ovarian cancer has the highest mortality rate. Additionally cervical cancer is tremendously increasing especially in low socioeconomic regions [1]. Initial staging surgery is an essential part of gynecologic cancers and adjuvant chemo/radio therapy, if indicated, shape the initial treatment modalities of these cancers [2].

Cytotoxic agents mainly affect rapidly proliferating cells. Although they are highly effective at the initial treatment process, as time passes they loss their effectiveness because of new mutations and other known/unknown processes of tumor cells. Recently new pathways for blocking tumor growth and dissemination are under research. Targeted therapy is an approach with direct and indirect methods. Direct method is altering tumor antigen signalling either by monoclonal antibodies (MoAbs) or by small molecule drugs on basis of target proteins. Indirect method is a ligand mediated way which are expressed on cell surface against tumor antigens [3]. In this manner, targeted therapy should be divided into three groups as antibody, small molecules and ligand-targeted therapy. So that, this treatment modality has a critical role on tumor growth, progression, invasion and metastasis by tumor cell selective therapy with less toxicity [4].

Some targeted therapies inhibit enzymes which take part in tumor growth and spread. Tyrosine kinase inhibitors, mTOR inhibitors, proteasome inhibitors, growth factor, signal-transduction and multikinase inhibitors are enzyme inhibitors of targeted therapy. Additionally inducing apoptosis and inhibiting angiogenesis are other mechanisms of targeted therapy.

Monoclonal antibodies

Monoclonal antibodies are relatively new innovations of cancer treatment. They are monospecific antibodies, which are produced by one type of immune cell in vitro and bind to specific areas in cancer cells. They recognize and bind to antigens and induce immune response after binding. In 1975 Kohler and Milstein found Hybridoma Technology to produce

large amounts of MoAbs [5]. First generation MoAbs (-momab) were murine, rabbit or rat proteins that were purified after immunization with an antigen. However patients produced antibodies against these foreign antigens as HAMA (human anti-mouse antibody) or HARA (human anti-rabbit antibody). These antibodies reduced the effectiveness of treatment and increased the risk of anaphylactic reactions. Whereas second generation MoAbs were produced with DNA technology; chimeric Abs (-ximab), humanized Abs (-zumab) and finally human Abs (-umab). MoAbs show their anti-tumor effect by cell-mediated cytotoxicity, complement dependent cytotoxicity, immunomodulation, altering signal transduction and immunoconjugates.

Small molecules

For regular cell life protein phosphorylation plays an important role and alterations in this process like mutations or over-expressions, have a role on tumor proliferation and angiogenesis [6]. Plasma membrane associated protein tyrosine kinases are the most studied group [7].

Ligand binding

There are many common features of tumor cells and normal healthy cells. However tumor cells are in a high metabolic state, and this helps cytotoxic agents to destroy tumor cells in a semi-selective method that is important for preventing side-effects to normal cells. In that manner, ligand mediated targeted therapy is effective[3] in limiting cytotoxicity and improve therapeutic efficacy of agents. Liposomal anti-cancer drugs work as ligand mediated therapy [8].

Molecular Basis of Ovarian Cancer and Therapeutic Targets

Ovarian cancer has the highest mortality rate among all gynecological cancers. Primary cytoreductive surgery with platinum based chemotherapy shape the initial management of these tumors. However, recently we are on the edge of molecular therapy for this cancer. In this setting we need to identify a dominant biological pathway for tumor progression and discover a functional and structural molecule within this pathway. In the literature, epithelial ovarian cancer has been treated as a single disease but now we know the heterogeneity related to the histological subtypes and its correlation with clinical outcomes. Defects in molecular

pathways; p53 for high grade serous tumors, BRCA for early familial serous cancers, BRAF, KRAS, NRAS, HER2 for low grade serous tumors, PIK3CA, PTEN for clear cell cancers and endometrioid tumors, KRAS and HER2 for mucinous cancers lead us towards specific targeted therapies [9].

Tumor vasculature and angiogenesis

Angiogenesis and development of new blood vessels have a crucial role in cancer growth and metastasis [10].

Epidermal growth factor (EGF) and Her2

The epidermal growth factor (EGF) receptor (EGFR) is a member of the Her family and is in relation with tyrosine kinase receptors. EGFR (ErbB1, Her1), Her2 (EGFR2, Her2/neu), Her3 (EGFR3, Erb3) and Her4 (EGFR4 or ErbB4) are the members of this family. After binding to a member of Her family receptor, it undergoes tyrosine phosphorylation. This goes on with the activation of pathways with PI3-kinase, MAP-kinase and other cytoplasmic signalling cascades [11]. EGFR is expressed in approximately 70% of cancers and it is associated with chemo-resistance, poor prognosis and advanced stage disease [12]. Activation of EGFR signalling increases proliferation, angiogenesis and decrease apoptosis. EGFR and Her2 are found in cancer cells. Overexpression of ERBB2 gene/HER2 occurs in approximately 20-30% of breast cancers [13]. Additionally it also occurs in ovarian and serous uterine tumors [14].

Vascular endothelial growth factor (VEGF)

Vascular endothelial growth factor (VEGF) and its receptors are critical in tumor progression. VEGF is mainly found in tumor microenvironment. VEGF binds to tyrosine kinase receptors to function. Activation of this pathway is related to MAPK and PI3/AKT cascades that effect endothelial cells [15]. Additionally VEGF increases vascular permeability and causes vasodilation that impairs delivery of oxygen and therapeutic agents to tumor cells [16]. Recently it has been shown that ovarian tumors express VEGF and its receptors (VEGFR) [17,18]. VEGF is also related with the development of malignant ascites [17]. Increased permeability and release of tumor cells into vascular tissue causes ascites formation and effusion. Platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) are other angiogenic pathways associated with ovarian cancer [19]. The humanized molecular antibody,

bevacizumab, which binds to circulating VEGF, is the most studied molecular therapeutic agent of these anti-angiogenic agents [20]. Preclinical data suggest benefits with bevacizumab as maintenance therapy after platinum based chemotherapy. Single agent bevacizumab inhibited or delayed disease recurrence and prolonged survival in murine ovarian cancer model [21]. Addition of bevacizumab to standard chemotherapy has improved progression free survival (PFS) in primary, recurrent platinum-sensitive and recurrent platinum-resistant ovarian cancer [22-24]. However there is a debate on the overall survival rates of ovarian cancer patients after bevacizumab. Initiation of bevacizumab for recurrent ovarian cancer has been studying in GOG 218 and ICON 7 trials are discussing bevacizumab as first line therapy with carboplatin and paclitaxel, then as maintenance. We know angiogenesis plays a role in the biologic behaviour of ovarian cancer; despite the truth of this statement clinical response rate to bevacizumab in recurrent ovarian cancer could not be predicted by tumor microvessel density [25]. Hypertension, bleeding, proteinuria, thromboembolism and gastrointestinal perforation are possible toxic effects of bevacizumab [26].

VEGF Trap

VEGF Trap is a fusion protein that is consisted with extracellular domains of VEGF-1 and VEGF-2. In mouse models VEGF Trap treatment decreased accumulation of ascites by vascular remodelling [27]. This agent may have a role in platinum resistant ovarian cancer; and it prolongs time to repeat paracentesis [16]. VEGF Trap is a promising agent for platinum resistant ovarian carcinoma patients with symptomatic ascites [28].

Tyrosine kinase inhibitors (TKIs)

Ovarian tumors overexpress VEGF, PDGF, angiopoietin and EGFR as proangiogenic factors [29]. Importantly VEGF is a highly studied molecule that omental metastatic tissue and ascites fluid of epithelial ovarian cancer patients enormously overexpress VEGF besides ovarian tumor cells [30]. Tyrosine kinases are catalytic enzymes that transfer gamma phosphate group from adenosine triphosphate to target proteins thus they have a pivotal role on normal cellular regulatory process. Tyrosine kinase inhibitors target growth factor signalling pathways. Small molecule inhibitors of tyrosine kinase compete with the ATP binding site of the catalytic domain at the extracellular and cytoplasmic region by the way they could decrease tumor cell proliferation, growth; induce apoptotic effects and blockade

angiogenesis and metastasis. The most important cytoplasmic signalling pathways are the phosphoinositide-3-kinase (PI3K)/AKT/mTOR pathway, the Ras/Raf mitogen-activated protein kinase (MAPK) pathway, the Raf/MEK/Erk pathway and the protein kinase C pathway [31,32]. BCR-ABL and KIT tyrosine kinase inhibitors (imatinib mesylate), tyrosine kinase inhibitors that target EGFR, VEGFR and PDGFR are also challenging therapeutic potentials for ovarian cancer.

They are orally bioavailable small molecule inhibitors that target especially angiogenesis and drawing a conclusion through small molecule tyrosine kinase inhibitors is difficult. There are numerous on-going studies. There are partial response rates for platinum sensitive and resistant ovarian cancer patients and moreover they will be provided for maintaining the disease at baseline stabilization [33-35]. Pazopanib is a well-known tyrosine kinase inhibitor that blockades VEGFR, PDGFR and c-KIT [36]. When CA-125 decline rate was considered as an objective criteria, Pazopanib showed 31% response rate after first or second line therapy [37]. It also maintained a high rate of PFS when compared with placebo in an on-going Phase III trial. Despite some benefits, administration of Pazopanib with chemotherapeutics can cause severe adverse effects [38].

A concern towards their toxicity profile should be taken for granted although they have a different cytotoxic model than conventional chemotherapies. Most common toxicities include gastrointestinal and hematological side effects as well as cutaneous hazards [34].

Folic acid receptor inhibitors

Folic acid has an essential role for one carbon transfer process in DNA synthesis additionally folate is crucial for nucleotide synthesis and methylation reactions. Cancer cells are strongly dependent on folate that highlights replication of DNA. Aminopterin, methotrexate and 5-fluorouracil are widely cited in the literature as chemotherapeutic agents for many cancers [38]. Folic acid analogues disrupt cellular proliferation by blocking one-carbon transfer and methylation reactions, additionally they form active polyglutamate derivatives to inhibit enzymes in folate metabolism [39]. Folate uptake is organised by folate receptors (FR) α and β . FR α (FOLR1) expression is mostly found in epithelial cells of uterus, placenta, choroid plexus, retina and kidney [40]. FR β expression is commonly restricted to placenta [41]. It has also been demonstrated that ovary, endometrium, breast and many other cancers overexpress

α folate receptors [42,43]. Most of serous ovarian cancers overexpress α -FR especially high grade, high stage cancers. Toffoli et al. [44] found 80% of epithelial ovarian cancers with FR α expression and stated the expression rate as a prognostic factor of stage and grade. FR α is likely to be a novel therapeutic target for primary and recurrent ovarian cancers. Although there is a tendency for earlier recurrence with FR α positive tumors, the effect of FR α receptor on survival rates is under debate in univariate and multivariate analyses [45]. Folate metabolism disruption by thymidylate inhibitors (pemetrexed), which inhibit thymidylate synthase that is required for DNA synthesis/ repair and farletuzumab, a monoclonal antibody targeting folate receptor, showed better PFS rates therefore simultaneous administration of chemotherapeutic agents with folate inhibitors has prolonged survival in Phase II trials [22] especially in platinum sensitive recurrent ovarian carcinoma patients [46].

Poly-ADP-Ribose Polymerase (PARP) inhibitors

PARP inhibitors are important agents for targeted therapy in ovarian cancer. These agents inhibit PARP enzyme. They are highly selective for homologous recombination (HR) deficiencies and their target is DNA repair process [47]. Inhibition of PARP enzyme leads to persistence of spontaneously occurring single-strand breaks (SSBs) and subsequently formation of double-strand breaks (DSBs) occur. This process intercepts repair of DSBs in cells with defective homologous recombination pathway, thereby cell death is ensured [48]. BRCA-1 and BRCA-2 germline mutation carriers are candidates for this treatment modality. Additionally they are related with high-grade serous cancers without these mutations [49]. 5-10% of ovarian carcinoma patients have a mutation in BRCA-1 and BRCA-2 gene; the lifetime risk of developing ovarian cancer for this gene mutation carriers is 40-50% and 10-20% respectively [50]. Olaparib, Niraparib and Rucaparib are examples of these drugs. They are administered orally. They are also in trials with platinum sensitive recurrent ovarian cancer patients with or without mutations. Nevertheless, in sporadic high grade serous ovarian cancers there are also defective genetic and epigenetic cascades in HR pathways; that might explain the usefulness of PARP inhibitors [51]. The overall response rate to PARP inhibitors is 30-40% however the real influence on progression free and overall survival is controversial on on-going studies [52,53]. Olaparib efficacy and platinum sensitivity is in association that clinical benefit rate of Olaparib in platinum refractory, resistant and sensitive patients is 23%, 45% and 69% respectively [54].

Aurora kinase inhibitors

Mitotic errors are commonly seen in tumor cells and they cause dysfunctional genomic state. Aurora kinases are in protein structure and they are one of the main mitotic protein kinases like Cdk1 (cyclin dependent kinase 1), Plk1 (Polo-like kinase) thus they regulate mitosis [55]. These proteins are highly expressed in proliferative cells [56]. There are three aurora kinases as A, B and C which take an active role during the G2-M phase of the cell cycle [50]. Aurora-A is especially important for centrosome functions [57] and Aurora-A is a potential oncogene [58] whose polymorphisms are related with 18-25% increased risk of ovarian cancer additionally breast or colon cancer [59,60]. Landen et al. [61] checked Aurora-A staining in ovarian cancer tissues after primary cytoreductive surgery and they found 82.9% of cases were having a staining greater than normal epithelium. Moreover Aurora kinase A overexpression is significantly associated with tumor grade, FIGO stage and survival [50]. Aurora kinase A is not only in association with tumor formation but also relevant to cancer progression and resistance to therapy [61]. Aurora kinases represent a potential molecular agent for ovarian cancer treatment; however developing a cytotoxic agent as Aurora kinase inhibition may not prevent their effects on non-tumor cells through mitotic ways. They may also act to blockade chemo-resistance and open areas for chemo-sensitivity [56]. Combination of taxanes or platinum containing chemotherapeutic agents with Aurora kinase inhibitors could produce synergistic effects and may increase cellular sensitivity to both chemotherapy regimens and radiation therapy by the way the resistant cells might decrease [62].

Hedgehog (Hh) signalling

Hedgehog pathway is a key mediator for the maintenance of stem or progenitor cells in many adult tissues that regulates differentiation, proliferation and homeostasis [63]. Hh pathway has a pivotal role on embryogenesis and it also has some functions in adult state. Hh proteins are signalling molecules and with their receptors they may cause disorganised and uncontrolled cell proliferation by mutations or over-expressions [64]. Hh signalling may take part in multiple steps during carcinogenesis. Activation of Hedgehog signalling could be detected approximately 30% of human cancers including ovarian cancers [65]. In mammals there are three Hh ligand proteins: Sonic hedgehog (SHh), Indian Hh and Desert Hh. Patched (Ptch) is a transmembrane protein receptor acts as a tumor suppressor which causes inhibition of

Smoothed (Smo) by the way of activated signalling cascades and blocks Gli-mediated transcription of target genes [65]. Increased expression of Shh, Patched, Smo and Gli1 proteins are seen in ovarian cancers [66]. Liao et al. [65] found a positive correlation between Patched, Smo expression and ovarian cancers; moreover they found overexpression of Gli1 and Patched protein as a negative indicator of poor prognosis and survival. However Yang et al. [67] showed a low percentage of Hedgehog signalling activation in ovarian cancers; they found 20% of tumor materials with detectable overexpression of two Hedgehog target genes. There are ongoing Phase I and II studies for Hedgehog inhibitors as a novel therapeutic for ovarian cancer remission.

PI3K-AKT-mTOR pathway inhibitors

The PI3K-AKT-mTOR pathway is an essential part of multiple functions in cell homeostasis that regulates cell proliferation, protein translation, cell metabolism and angiogenesis [68]. The PI3K-PTEN-AKT-mTOR pathway is a major signalling downstream of tyrosine kinase receptors [69]. Genetic amplifications and mutations of PI3K could be detected in ovarian and other gynecologic cancers. They can lead to oncogenic transmission through activation of AKT which upregulates mTOR activity [70]. Inhibition of mTOR causes interruption in cell cycle progression and induces an arrest at G1 phase of cell proliferation [71]. By the way mTOR regulates phosphatidylinositol-3-kinase (PI3K)/AKT signalling pathway.

For cancer development; mTOR signalling pathway is in association with many fundamental molecules in cell survival like PI3K amplification/mutation, AKT overexpression, loss of PTEN, loss of P53 function and overexpression of S6K1 [72]. Mutations of P53 and PTEN are commonly detected in human cancers and target of mTOR inhibition is partially beneficial for this type of tumors [73]. In tumor cells hypoxia is a frequent finding that hypoxia inducible transcription factor (HIF) and expression of other growth factors such as angiopoietin 1 and 2 (ANG 1, ANG 2), basic fibroblast growth factor (bFGF), tumor growth factor- β (TGF β) and platelet derived growth factor (PDGF) came into existence because of hypoxia. These factors activate PI3K/AKT/mTOR in many cells [74]. mTOR induces two distinct molecules mTORC1 and mTORC2; mTORC1 activates S6K1, 4E-BP1, eIF4E and mTORC2 activates PKC- α , SGK1 thus they functionalize protein translation/cellular proliferation and cellular metabolism respectively [72]. Inactivation of mTOR can block G0-

G1 cell cycle so that inhibition of tumor cell growth occurs. AKT activation prevents cells from apoptosis thus AKT/mTOR is a potentially important target for therapeutic and chemopreventive effects of agents [75]. Additionally activation of AKT and PI3K could take part in resistance to chemotherapy [76,77].

Conclusion

The new therapeutic modalities with targeted molecules should build up new perspectives insight tumor cell behaviour. Agents against angiogenesis and receptors of growth factors in addition to signalling cascades and intracellular enzymes could shape the individual treatment protocols for cancer survivors. These hopeful studies are ongoing and in near future what we know and do could totally change.

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