Principles of Chemotherapy, Targeted Therapy, and Immunotherapy in Gynaecological Malignancies

Satya Sadhan Sarangi, Sudeep Gupta, and Amal Chandra Kataki

1 Introduction

In the management of cancer, both surgery and radiotherapy are essential local forms of treatments that are directed towards primary tumours and any loco-regional disease. Chemotherapy is a systemic modality and can treat distant metastases. Chemotherapy is used to improve the prognosis in majority of cancers, but is curative only in the minority of cancers. Chemotherapy is well known to cure lymphomas, leukemias, testicular cancers, and choriocarcinoma.

The main aim of delivering anti-neoplastic drugs is to eradicate cancer cells without causing excessive toxicity to normal cells. But anti-neoplastic drugs have considerable toxicity as it cannot easily differentiate between malignant cells from normal cells.

S. S. Sarangi (✉)
Department of Medical Oncology, Dr. Bhubaneswar Borooah Cancer Institute (A Unit of Tata Memorial Centre, Mumbai), Guwahati, Assam, India

S. Gupta
ACTREC (Advanced Centre for Treatment, Research and Education in Cancer), Navi Mumbai, Maharashtra, India
e-mail: sudeep.gupta@actrec.gov.in

A. C. Kataki
Department of Gynaecologic Oncology, Dr. Bhubaneswar Borooah Cancer Institute (A Unit of Tata Memorial Centre, Mumbai), Guwahati, Assam, India

2 Basic Principles

Chemotherapy agents are grouped into different categories based on the mechanism of action.

These categories include:

1. Alkylating agents
2. Topoisomerase inhibitors
3. Antimicrotubule agents
4. Antimetabolites
5. Plant alkaloids
6. Anthracyclines

In addition to the site of primary tumour and diagnosis, individual patient-related factors such as organ function, age, performance status, concurrent medical illness, and residual toxicities from the receipt of prior therapies also influence the selection of chemotherapy regimens. Depending on the goals of treatment and previous treatments the patient may require dose adjustments. Treating oncologist who is prescribing anticancer agents should understand the intent of care for the individual patient (curative vs. palliative) as well as the metabolism and toxicities of the chemotherapeutic agents prescribed. Patients and their families should be informed about the expected toxicities and aim of therapy.

A. C. Kataki, D. Barmon (eds.), *Fundamentals in Gynaecologic Malignancy*,
https://doi.org/10.1007/978-981-19-5860-1_7

3 Growth of Tumour Cell

Tumour growth is a complex and intricate process that is governed by genetic abnormalities within the cell and the interaction of tumour with its microenvironment. The understanding of cancer has accelerated significantly over the past decade, and Hanahan and Weinberg [1] have defined the distinguishing features of cancer detailing the following hallmarks in addition to genomic instability as an underlying premise of the make-up of cancer cells:

1. Promotion and sustaining proliferative signaling,
2. Activating invasion and metastasis,
3. Resisting cell death,
4. Allowing replicative immortality,
5. Induction of angiogenesis,
6. Evading growth suppressors,
7. Immune surveillance evasion,
8. Altered Energy reprogramming.

The proliferation and growth control of normal cells are not well understood, but the mitogenic signaling of cancer cells is increasingly better understood. Cancer cells acquire the ability to proliferate unchecked by several different mechanisms: self-production of growth factor ligands; control of the tumour microenvironment by signaling local stromal cells, which in turn produce factors leading to cancer growth; overexpression or enhanced signaling of transmembrane receptors; and growth factor independence via constitutive activation of tyrosine kinases within the receptor and/or downstream signaling molecules [2]. Enabling characteristics of cancer cells that allow the above changes to occur include overall genomic instability and the cancer cell's ability to avoid immune destruction [3].

4 Log Kill Hypothesis

Cell kinetics was originally described based on murine models, but later on it was seen that most human solid tumours do not grow exponentially. The log kill hypothesis was based on the L1210 murine leukemia model, which is fast-growing leukemia where 100% of the cells are actively progressing through the cell cycle [4]. Logarithmic kill hypothesis states that a given anticancer drug should kill a constant proportion or fraction of cells in contrast to a constant number of cells, and cell kill is proportional regardless of the bulk of tumour. For example, if a drug can lead to a 3 log kill of cancer cells and can reduce the cancer burden from 10^9 to 10^6, the same drug and dose can also reduce the tumour burden from 10^6 to 10^3. However, solid tumours tend to follow the Gompertzian model of tumour growth because most solid tumours do not grow and expand exponentially [5]. The Gompertzian model (Fig. 1) predicts that cell growth is faster at the start of the growth curve when a tumour is small compared to a larger tumour existing in the slower part of the growth curve, which thus has a lower growth fraction. The Gompertzian model also predicts that the sensitivity of cancer to chemotherapy depends on where the tumour is in its growth phase and that growth decreases exponentially over time. Similarly, the log kill produced by chemotherapy is higher in small-volume tumours than large-volume tumours because of the differences in growth kinetics.

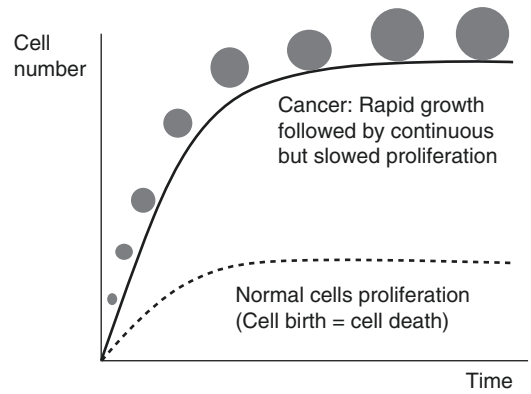

Fig. 1 Gompertzian growth curve. (Adapted with Permission from 2014 Pan Stanford Publishing Pte. Ltd through email communication)

5 Resistance to Chemotherapy

Resistance occurs with all cancers except for those that are curable. Multiple mechanisms are there, new mechanisms are also being discovered, and overlapping mechanisms can occur in tandem; tumour resistance to drug therapy results primarily from tumour growth and selection of existing resistant clones while sensitive cells are killed [6, 7]. One of the original hypotheses explaining drug resistance is the Goldie and Coldman hypothesis reported initially in 1979, which served as the basis for drug regimens used in hematologic malignancies and more recently in gynaecologic malignancies [8]. The tenets of the Goldie and Coldman hypothesis include the following:

1. Treatment should be started as soon as possible to treat the smallest amount and bulk of the tumour,
2. Multiple non-cross-resistant drugs should be used to avoid selection of resistant clones, and
3. Drugs should be used as frequently as possible and in doses that are higher than minimally cytotoxic doses.

In clinical trials that examine features of the Goldie and Coldman hypothesis, adjuvant breast cancer therapy has shown improvements in outcome by using this theory, but in the upfront treatment of ovarian cancer, the use of sequencing non-cross-resistant agents did not result in improved progression-free survival or overall survival [9]. Examples of mechanisms of drug resistance include alteration of drug movement across the cell membrane with respect to both influx and efflux, increased repair of DNA to offset damage done by certain agents, defective apoptosis so cancer cells are not receptive to drug effects, alteration of drug targets such as topoisomerase II alteration by point mutation, deletions or overexpression, and other mechanisms. The mechanisms of resistance associated with specific agents are discussed within the individual drug descriptions [6, 10]. Newly described drug resistance mechanisms include the identification of secondary mutations that restore the wildtype BRCA reading frame, which is likely a mediator of acquired resistance to platinum-based chemotherapy [11].

6 Dose Intensity

The therapeutic selectivity of chemotherapy is reliant on the outcome of dose-response between normal tissue and cancer tissue. Dose intensity is the amount of drug delivered per unit of time, and the dose intensity of each regimen is based on the time period during which the treatment is administered. Calculations can be made regarding the intended dose intensity as well as the actual dose intensity that the patient receives in total. By reducing the dose intensity to decrease toxicity, clinicians may compromise the predicted outcome of a patient, and therefore, clinicians must state the upfront intended outcome of administering chemotherapy (i.e., curative vs. palliative). The importance of maintaining dose intensity has been demonstrated in early-stage breast cancer patients using adjuvant cyclophosphamide, methotrexate, and 5-fluorouracil, as well as cyclophosphamide and doxorubicin. In gynaecologic cancers, the importance of dose intensity has been observed in older patients with ovarian cancer who may have worse outcomes compared to younger patients because of reduced dose intensity and less aggressive dosing of chemotherapy in older patients [12]. In another example, EMA-CO regimen is the most commonly used combination chemotherapy among other regimens that have been used in the management of high-risk GTN. Combination chemotherapy is often administered at 2- to 3-week intervals and timely administration is essential. Unnecessary treatment delays and dose reductions should be avoided as they may lead to tumour resistance and treatment failure. Several mechanisms to deliver chemotherapy in a dose-intense fashion are available to clinicians. First, doses of drugs can simply be escalated. Second, the same doses of drugs can be given in a reduced interval of time (i.e., "dose-dense administration"). For example, adjuvant cyclophosphamide and doxorubicin followed by paclitaxel in early breast cancer administered every 2 weeks rather than every 3 weeks demon-

strated improvements in the dose-dense regimen [13]. The prophylactic use of growth factors has enabled chemotherapy to be delivered at higher doses safely without excess risk of neutropenic events and has enabled chemotherapy to be delivered in a dose-dense manner.

7 Single Versus Combination Therapy

Decisions regarding optimal choice of single agent versus combination therapy should be based on the objectives of therapy (curative vs. palliative treatment), published regimens for specific indications and dosing of agents, and predicted toxicities. Specific doses chosen should be based on published studies, but dose alterations can occur based on objectives of treatment; renal, hepatic, or bone marrow function; toxicities experienced by the patient during previous cycles; current performance status and comorbidities of the patient; direct measurement of drug levels in the individual patient when possible; and potential interactions with other concomitant medications. Although combination chemotherapy typically yields higher response rates overall compared to single agents, toxicities are usually higher; outcomes such as overall survival and progression-free survival may be better with combinations [14]. Scheduling of drugs is such that the most myelosuppressive agents are given on day 1 and scheduled every 2–4 weeks depending on the timing of the myelosuppression nadir. This allows for the recovery of bone marrow, gastrointestinal, dermatologic, and other organ toxicities without allowing significant tumour growth to occur. Mechanisms of action of the drugs and duration of infusion can also influence drug sequencing and toxicities.

8 Different Chemotherapy Types

Primary Therapy In which chemotherapy is the primary modality of treatment, e.g. Methotrexate in treatment of choriocarcinoma.

Adjuvant Therapy It is defined as the usage of systemic chemotherapy after surgery and/or radiotherapy with radical intent in patients who have subsequent high risk of recurrence.

Concurrent Chemoradiation Usage of chemotherapy in combination with radiation with curative intent to sensitize the tumour cell. Chemotherapy is used during radiation therapy to eradicate micrometastases within the radiation field and to increase the radio-responsiveness of tumour cells. Regimens using alternate radiation and chemotherapy aims to reduce toxicity to normal tissues and enhance tumour sensitivity by delivering each agent when the first agent has induced enhanced sensitivity to the other. The initial reduction of the tumour mass by chemotherapy results in improved tumour's blood supply, thus improving re-oxygenation, and improving radiation-induced tumour cell kill. Chemotherapy also plays an essential role in the segregation of the tumour cells in a favorable manner, permitting radiation to be more effective in a particular phase of the cell cycle. Conversely, radiation therapy may decrease the tumour mass, leading to improved blood supply [15] and optimal drug delivery. Few cancer chemotherapy agents function as radiation sensitizers, showing synergistic cancer cell kill when combined with radiation while having a lesser relative side effect on normal tissues.

Neoadjuvant Chemotherapy Defined as the use of chemotherapy in the treatment of the locally advanced disease that will assist in subsequent definitive treatment.

Induction Chemotherapy Initial systemic chemotherapy used in patients with the disseminated disease for which local modality (i.e., surgical resection or radiotherapy) is incomplete or not indicated.

Salvage Chemotherapy When chemotherapy is given for recurrence after initial treatment with chemotherapy or second-line chemotherapy if it does not respond to initial induction therapy. Generally, the intent is palliation.

Consolidation Chemotherapy Additional chemotherapy for more than the usual regimen.

Palliative Chemotherapy Chemotherapy when it is used for symptomatic relief in incurable cancer.

9 Routes of Administration

Intravenous Most chemotherapeutic agents are available only in an intravenous preparation, requiring venous access. Venous access can be temporary during chemotherapy administration or prolonged by surgically implanted intravascular devices, type of access must be chosen carefully for the anticipated duration, complexity, types of drugs (vesicants or non-vesicants), and anticipated need of fluid replacement, blood products, and antibiotics. Patient preference and quality of life issues also play a role.

Oral and Local Drug application intra-arterial (i.e., hepatic infusion, limb perfusion), intrathecal (meningeal metastasis), intraperitoneal (ovarian cancer, peritoneal carcinomatosis), intrapleural (pleurisy/pleural metastases), and intrapericardial (malignant pericardial effusion) are other routes of administration. Various agents are available in oral form, making intravenous access unnecessary. Besides their everyday use in various chemotherapy regimens, oral agents, have ease and convenience, also play a vital role in palliative therapy [16], where the quality of life issues are paramount. The use of oral agents is to be restricted in patients with functional or anatomical barriers for ingestion and absorption.

Intraperitoneal Therapy Direct intraperitoneal instillation of chemotherapy drugs provides a two- to five-fold and higher concentration advantage over systemic intravenous administration. It exposes tumour cells to both higher peak drug concentrations and area under the [17] concentration-time curve drug levels. Intraperitoneal therapy is typically accomplished through a surgically implanted intraperitoneal catheter, which may be either exteriorized or subcutaneous. Intraperitoneal therapy is best used to treat small-volume, diffuse intraperitoneal [18] disease, and has thus been used mainly in ovarian cancer, with less common application in intraperitoneal [19] gastrointestinal malignancies or mesothelioma. Drugs such as cisplatin, carboplatin, 5-fluorouracil (5-FU), and paclitaxel are used in intraperitoneal therapy.

10 Chemotherapeutic Drugs Used in the Treatment of Gynaecological Cancer

Majority of anticancer drugs cause damage to DNA with the help of various mechanisms, including disruption of cell cycle checkpoints, growth factors, growth factor receptors, and signal transduction.

10.1 Alkylating Agents

Alkylating agents are considered as one of the earliest chemotherapy agents that started to be

used in the early 1940s. These agents block DNA replication by forming covalent bonds with DNA bases (cross-linking the DNA strands). Examples are cyclophosphamide, ifosfamide, melphalan, procarbazine, busulfan, etc. Chlorambucil can be given by mouth and is the least toxic. Cyclophosphamide and ifosfamide produces an acrolein metabolite that causes hemorrhagic cystitis. Use of mesna and hydration has largely overcome the hemorrhagic cystitis. Mesna inactivates the acrolein metabolites in the urine and is rapidly excreted in the urine. Cyclophosphamide can be used in patients with renal impairment. However, ifosfamide can cause cumulative renal tubular damage and resulting in Fanconi syndrome.

10.2 Platinum

The first platinum-based chemotherapy drug is cisplatin that was approved in 1978. Platinum can be considered as the most important chemotherapy drug used in gynaecological cancer. Platinum compounds form DNA cross-links by an action similar to the alkylating agents. Cisplatin, carboplatin, and recently oxaliplatin are platinum-based cytotoxic drugs. Cisplatin has more nephrotoxicity and ototoxicity than carboplatin. Carboplatin is a second generation and oxaliplatin is the third-generation platinum-based cytotoxic agent.

10.3 Anti-tumour Antibiotics and Anthracylines

Anti-tumour antibiotics have a variety of modes of action, including DNA cross-linking and topoisomerase inhibition. Some of these drugs intercalate between base pairs of DNA and RNA, inhibit RNA and DNA polymerase, generate oxygen-free radicals, and alter cell membrane function. The most important and commonly used members of this drug are doxorubicin and epirubicin. Doxorubicin is isolated from a mutated strain of streptomyces. Actinomycin D is also anti-tumour antibiotic (polypeptide antibiotic), which is isolated from the bacteria of the genus streptomyces. Doxorubicin, epirubicin, and idarubicin are also known as anthracycline drugs (anthracycline antibiotics). The short-term dose-limiting side effects of anthracycline drugs are myelosuppression and mucositis. Cardiomyopathy is also a dose-limiting side effect and can be prevented by controlling the total dose and the use of chemoprotection cardioxane (dexrazoxane) before treatment. Liposomal doxorubicin is the customized form of doxorubicin to enhance efficacy and reduce toxicity. The doxorubicin is encapsulated inside the liposome; liposome allows the doxorubicin to remain in the body for a longer duration. Encapsulated doxorubicin is also considered to reduce exposure of the active metabolite to myocardial tissue and therefore, decreasing myocardial toxicity. Currently, there are two types of liposomal doxorubicin available (differ in their lipid component), i.e. pegylated liposomal doxorubicin hydrochloride and Myocet. Myocet is indicated as a combination with cyclophosphamide in metastatic breast cancer. Bleomycin is also an anti-tumour antibiotic, discovered in the 1960s. Bleomycin acts by creating free oxygen radicals which break DNA strands, similar to anthracycline. These drugs are commonly used in the treatment of breast cancer, uterine sarcoma, and ovarian cancer.

10.4 Antimetabolites

Antimetabolites are cytotoxic agents which have structurally resemblance with naturally occurring purines, pyrimidines, and nucleic acids. They inhibit the key enzymes which are involved in DNA synthesis. They can add into the DNA or

RNA of the cancer cells and interfere with the cell division process. Examples are Methotrexate, 5-fluorouracil, 6-mercaptopurine, gemcitabine, etc. Methotrexate is an anti-folate drug, which is structurally similar to folic acid and inhibits the activity of DHFR enzyme leading to inhibition of DNA and RNA synthesis. Gemcitabine is a deoxycytidine analogue, a pyrimidine antimetabolite related to cytarabine. Gemcitabine is a pro-drug and metabolized intracellularly to the active forms known as dFdCDP and dFdCTP. Both active metabolites will incorporate into DNA and inhibit DNA synthesis resulting in apoptosis.

10.5 Vinca Alkaloids

Vinca alkaloids are plant derivatives (from the periwinkle plant), Vinca rosea (catharanthus roseus) that have been traditionally used by the natives of Madagascar to treat diabetes. Vinca alkaloids falls into the category of tubulin-binding drugs jointly with taxanes. Examples are vincristine, vinblastine, vinorelbine, and vindesine. Vinblastine and vincristine bind to tubulin dimers and prevent their assembly into microtubules. These are highly vesicant. This should be injected into a central venous line or cannula where there is no resistance to injection and where blood can be freely drawn back. Vinorelbine is a synthetic vinca alkaloid which is available both intravenously and orally.

10.6 Topoisomerase Inhibitors

Topoisomerase inhibitors (I and II) are a group of enzymes which allow unwinding and uncoiling of supercoiled DNA. Both Topoisomerase inhibitor I and II act by interfering with DNA transcription, replication, and function to prevent DNA supercoiling. Topoisomerase I inhibitors are extracted from the bark and wood of the Camptotheca accuminata, they form a complex with topoisomerase DNA. Topotecan and Irinotecan are topoisomerase inhibitor I. Topoisomerase II inhibitors are extracted from the alkaloids found in the roots of May Apple plants. Topoisomerase II enzyme binds covalently to complementary strands of double-strand DNA, cleaving both strands. The inhibitors of this enzyme will reseal these breaks. Examples are etoposide, teniposide, and amsacrine. They are also classified under epipodophyllotoxins chemotherapy agents. Hematological toxicity are the main side effects of topoisomerase inhibitors. These are excreted by the liver and renal tubules; hence, dose adjustment may be necessary with renal and hepatic impairment.

10.7 Taxanes

Taxanes are plant alkaloids which were initially developed for therapeutic use in 1963. These are extracted from the yew tree. Paclitaxel is the first taxane to be discovered in 1971 and was made available for clinical use in 1993. Paclitaxel is isolated from the bark of the Western Pacific yew tree. Docetaxel is the semi-synthetic second generation of taxanes derived from the needles of the European yew trees. Both drugs work in the M-phase of the cell cycle and stop the function of microtubules by binding with them resulting in a sustained block in mitosis. Summary of mecha-

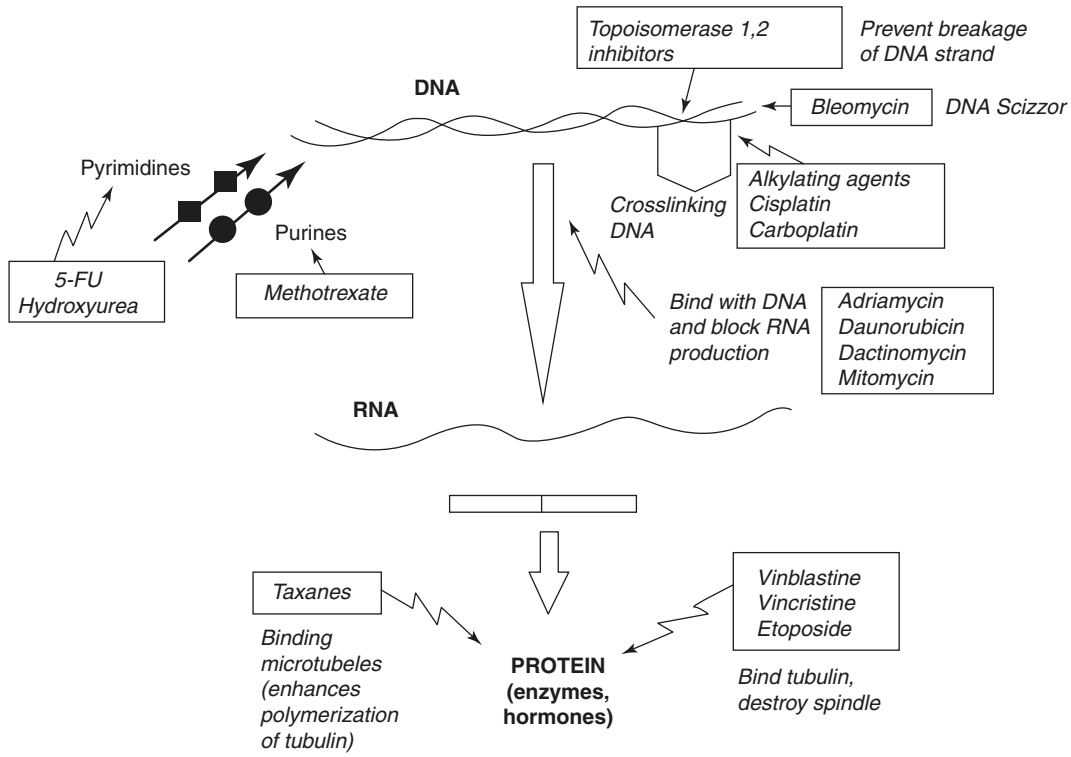

Fig. 2 Mode of action of chemotherapy agents used in Gynaecological Malignancies. (Adapted with Permission from 2014 Pan Stanford Publishing Pte. Ltd through email communication)

nism of action of different chemotherapeutic agents are shown in Fig. 2.

11 Targeted Therapies

The principal tenets of optimal disease management in women with gynaecologic malignancies have been the strategic utilization of surgery, cytotoxic chemotherapy, hormonal therapy, and radiotherapy. Although substantial progress has been realized from these practices, disease-specific mortality from gynaecologic malignancies still accounts for about 9% of all cancer-related deaths and underscores the need for the development of new therapeutic modalities. Investigation into the mechanisms governing cancer initiation, proliferation, metastases, autophagy, and apoptosis have uncovered a wealth of new opportunities, many of which harbor the potential of reversing the malignant phenotype, selectively inducing cancer cell death, overcoming primary and induced drug resistance, and optimistically improving overall outcomes for patients. The ability to pharmacologically and pharmacodynamically interact with these new "targets" has fostered rapid drug development, some of which is beginning to show merit in the treatment of women with gynaecologic malignancy. Because the biology of cancer growth often shares homology across different tumour types, targeted therapies are being investigated where the pathway of aberration is suspected to play an important or dominant role in disease pathogenesis. Although an "Achilles' heel," or a solitary activated pathway, is not present in most solid tumours, the opportunity to selectively target key regulatory and survival mechanisms in the tumour microenvironment holds great promise in expanding our

therapeutic armamentarium for these women. We review some of these pathways and agents in this section.

11.1 Mechanisms of Action

One of the most common events defining the cancer process is the dysregulation of protein kinases that govern normal cellular function. In light of this observation, proteins are frequently the targets of anticancer agents. Although there are many ways to affect protein kinase function, including small molecules, monoclonal antibodies, antagomirs, antisense, RNA interference, immuno- and receptor drug conjugates, decoy receptors, allosteric inhibitors, and nanotubes, the intent is to target these aberrancies either restoring normal host function or inducing cell death. The principle challenge is to affect tumour cells without impacting the function of normal host cells. Three relevant mechanisms are important to review.

11.2 Interruption of Signal Transduction Pathways

Signal transduction is the process where a ligand, usually lipophobic (e.g., a growth factor), meets a receptor or channel on the cell surface and initiates a cascade of events such as kinase activity or dissociation of G-coupled proteins resulting in some cellular response. In contrast, lipophilic ligands (e.g., steroids) can penetrate the cell membrane and may affect cellular functions by direct binding to cytoplasmic or nuclear targets. Many of the "small molecules" being developed for cancer therapy involve blocking the tyrosine kinase activity of membrane-bound receptors that are usually influenced by a number of promoting ligands. The prototypical example of a relevant ligand-receptor signal transduction pathway in carcinogenesis is the epidermal growth factor receptor (EGFR). This receptor family is overexpressed and activated in many tumour types, including gynaecologic malignancies, and appears to play a key role in disease pathogenesis. Binding of the epidermal growth factor (EGF) ligand to the receptor induces tyrosine kinase activity, which leads to receptor dimerization and activation of the pathway driving multiple cellular functions such as cellular proliferation, enhanced cellular motility, resistance to apoptosis, and angiogenesis. Because of the broad spectra of activity, there has been intense interest in developing therapeutics against this pathway. Typically, these targeted agents are classified in two broad categories: competitive adenosine triphosphate (ATP)–pocket small-molecule inhibitors and monoclonal antibodies to the receptor's extracellular domain. The clinical experience of these molecules in gynaecologic cancer will be discussed later; however, the crafted directive of these targeted agents is to disrupt ligand/receptor activation in the hopes of blocking the signal transduction pathways leading to cancer cell survival. This receptor family is overexpressed and activated in many tumour types, including gynaecologic malignancies, and appears to play a key role in disease pathogenesis [20].

11.3 Induction of Apoptosis

Normal development and functional physiology are dependent on tight regulation of cellular growth and death. The representation of cancer as "uncontrolled cellular proliferation" attests to the importance dysregulated cellular programmed cell death, or apoptosis plays in human disease. Phenotypical transformation of a normal cell to a cancer cell is likely highly influenced by the loss of apoptotic function. In addition, resistance to chemotherapy-induced cytotoxicity is frequently the result of cellular escape from apoptotic inducement. Two dominant pathways govern cellular apoptosis: extrinsic, induced via a receptor–ligand interaction (death receptor), and intrinsic, induced via mitochondria-apoptosome signaling. The converging points for both pathways are the effector caspases, which are closely regulated by upstream signaling proteins either inducing apoptosis or preventing it. A caspase-independent pathway also exists and appears to be mediated through apoptosis-inducing factor (AIF), which

is released from mitochondrial pores under control of Bcl-2 and induces nuclear chromatin clumping. The ultimate declaration of apoptosis is largely the balance of proapoptotic proteins (BAX, BID, BAK, and BAD) and anti-apoptotic proteins (Bcl-XI and Bcl-2). Numerous ligands have been identified as substrates for the death receptor including TNF, TNF-related apoptosis-inducing ligand (TRAIL), and Fas. Recently, novel targeted agents harboring agonist activation of this pathway at both the ligand and receptor levels have entered clinical trials [21]. p53, the most commonly mutated gene in human malignancy, functions as a transcription factor regulating downstream genes involved in DNA repair, cell cycle arrest, and both the intrinsic and extrinsic apoptotic pathways. p53, when activated, promotes the proapoptotic genes of the BCL-2 family, which inhibit Bcl-2 at the mitochondrial membrane, as well as activate expression of the death receptors, such as DR5 [21]. In this manner, cross-talk between the intrinsic and extrinsic pathways is extensive. When p53 is dysfunctional, one or both of these pathways may drive carcinogenesis; thus, this serves as a rationale to consider combinatorial treatment approaches, such as targeted therapy of the death receptor ligand in combination with cytotoxic chemotherapy.

11.4 Stimulation of the Immune Response

The immune system is a highly complex and interactive network of specialized cells and organs working in conjunction to maintain health. It is of no surprise that attempts at leveraging innate response or inducing a heightened response to cancer cells have been the subject of cancer therapeutic investigation for decades. The slow, albeit measured, clinical progress in this regard is a reflection of the complexity of the system, the evasiveness of cancer cells, and the imperfect models to preclinically study the system. However, the efficiency, selectivity, and sensitivity of the immune response make it one of the most promising avenues of targeted therapy and worthy of the effort. Key effectors of the immune response include cytokines, such as interferons and interleukins, and antibodies. Contemporary understanding of the interplay between cancer and the immune system suggests that although cancer cells are immunogenic, they do not always elicit a response. This "immunotolerance" is not well understood but may be mediated in part by local anti-inflammatory tumour cytokine production, which may prevent dendritic cells from properly processing tumour cell antigens for a robust immune, anticancer response. Nevertheless, several avenues of investigation have been pursued, the agents used in this regard are called biologic response modifiers (BRMs). The first BRMs to be created and used in cancer therapy were the interferons. As discussed earlier, this class of compounds has both direct and indirect activity on cancer cells. For example, the interferons can slow cancer cell growth or induce phenotypic transformation into normal cell behavior. Interferons also stimulate NK cells, T-cells, and macrophages, which may increase the efficiency of the immune response to effect better anticancer treatment. Several interferon compounds (α, β, and γ) have been US Food and Drug Administration (FDA) approved for cancer therapy, and many have entered mature clinical investigation, including for gynaecologic cancers, albeit with mixed results. For instance, an Austrian phase 3 study randomized 148 women with International Federation of Gynaecology and Obstetrics stage IC-IIIC disease to cisplatin/cyclophosphamide with or without subcutaneously administered interferon-γ. PFS at 3 years was significantly improved (17 vs. 48 months; Relative Risk (RR), 0.48; 95% CI, 0.28–0.82), and toxicity was considered comparable between the arms. However, a much larger phase 3 study conducted by the GRACES clinical trial consortium investigating combination paclitaxel/carboplatin with or without interferon-γ-1b in women with advanced-stage ovarian cancer was terminated early due to an interim futility analysis suggesting detrimental effects in the experimental cohort [22]. The second class of

cytokines being investigated as cancer therapeutics is the interleukins (IL). These naturally occurring families of compounds have a vast cache of activities in multiple host systems, including lymphoproliferative organs and angiogenesis and immune system effectors, such as lymphocytes and platelets. Currently, IL-2 (aldesleukin), an IL that stimulates growth and differentiation of the T-cell response, is FDA approved for the treatment of metastatic renal cell carcinoma and melanoma. However, in light of the numerous functions ILs drive in the immune and host response to cancer cells, investigators continue to search for key treatment opportunities. For example, it has been known that IL-6, a proinflammatory cytokine that impacts hematopoietic stem cells, is a poor prognostic factor (associated with advanced disease, chemotherapy resistance, early recurrence, and short survival) of several solid tumours, including the gynaecologic cancers, and is closely linked to angiogenesis, particularly in ovarian cancer, where high levels are also identified in ascites [23]. It also may be an important mediator of the paraneoplastic thrombocytosis phenotype, which is commonly identified in patients with advanced-stage ovarian cancer.

Molecular pathways most commonly targeted in solid tumours are:-

(a) Epidermal growth factor receptors (EGFR),
(b) Vascular endothelial growth factors (VEGF),
(c) HER-2/neu.

The following pathways can be repressed at multi-levels: (a) binding and neutralizing ligands, (b) occupying receptor-binding sites, (c) blocking receptor signaling within the cancer cell, and (d) interfering with downstream intracellular molecules.

There are two types of targeted agents:

(a) Monoclonal antibody with larger molecules (large-molecule inhibitors): It targets extracellular component such as ligand and receptor. The route of administration of this group is intravenous. The examples of large-molecule inhibitors are bevacizumab, trastuzumab, alemtuzumab, cetuximab, gemtuzumab ozogamicin, panitumumab, and rituximab.
(b) Small-molecule inhibitors: They enter cells and inhibit receptor signaling (mainly tyrosine kinase) and interfering with downstream intracellular molecules. Tyrosine kinase signaling initiates a molecular cascade that leads to cell growth, proliferation, migration, and angiogenesis in normal and malignant tissues. As compared to large-molecule inhibitors, small-molecule inhibitors are commonly administered in oral form and are cheaper than large-molecule inhibitors. The examples of small-molecule inhibitors are bortezomib, dasatinib, erlotinib, gefitinib, and others such as imatinib, lapatinib, sorafenib, and sunitinib. Epidermal growth factor receptors are also present in normal cells; therefore, EGFR inhibitors can cause dermatologic complications such as skin rashes and gastrointestinal complications such as diarrhea and abdominal pain.

Anti-VEGF is also known as anti-angiogenesis; without new vessel formation, the tumour cannot grow. Due to better blood supply in the remaining tumour, the delivery of anticancer drugs will be more efficient. However, anti-VEGF will also affect normal blood vessels leading to bleeding, thrombosis, and proteinuria due to alteration in glomerular infiltration, bowel perforation, and hypertension. Bevacizumab is a humanized MoAb against VEGFA that is approved by the FDA for the treatment of metastatic colorectal, non-small-cell lung, renal cell, and breast cancers. Several phase 2 trials of this VEGFA antibody have been performed to assess its activity in gynaecologic cancers. Bevacizumab has been most extensively studied in recurrent ovarian cancer patients, where response rates have ranged from 16% to 24% and median overall survival is 10.7–17 months when administered either as a single agent or in combination with metronomic cyclophosphamide [24–26]. It has also been shown to have activity in the patient

with recurrent or persistent endometrial cancer and patients with progressive or recurrent cervical cancer [27, 28]. Majority studies of bevacizumab in gynaecologic cancer have been performed in patients with recurrent or progressive disease. Following encouraging data in phase 2 studies compared with historical controls, two randomized phase 3 studies in untreated advanced ovarian cancer patients have been conducted: GOG 218 (NCT00262847) and ICON-7 (NCT00483782). Each of these trials included an experimental arm with a maintenance treatment phase, which was placebo-controlled in GOG 218 and open label in ICON7. Both trials showed enhanced clinical activity (hazard for progression) over control and, in the case of GOG 218, over combination of paclitaxel, carboplatin, and bevacizumab followed by placebo maintenance. Of interest, the PFS of these "winning" arms is substantively less than that reported by earlier phase 2 data despite a similar proportion of suboptimal stage IIIC patients.

11.5 PI3K/mTOR/Akt Pathway

PTEN (phosphate and tensin homolog detected on chromosome 10) is a tumour suppressor gene, which is important for normal cellular function. Mutations in PTEN can cause decreased apoptosis and these are seen in up to 83% of endometrioid carcinomas of the uterus. Due to mutation, there is decreased transcription which leads to less phosphatidylinositol 3-kinase (PI3K) inhibition, enhanced activity of Akt, and uncontrolled function of mammalian target of rapamycin (mTOR). Elevated activity of mTOR is seen in a vast majority of endometrial cancers as well as approximately 50% of cervical adenocarcinomas and 55% of ovarian carcinomas [29]. mTOR is a kinase that regulates cell growth and apoptosis [30]. Temsirolimus, ridaforolimus, and everolimus are mTOR inhibitors that have been tested as single agents in phase 2 studies and found to promote stable disease in 44% of patients with metastatic or recurrent cancer of the endometrium [31]. Myelosuppression, hyperlipidemia, hypercholesterolemia, and fatigue are most commonly seen side effects of these drugs. Because aberrations in the PI3K/Akt/mTOR pathway are prolific in gynaecologic cancers, drug discovery is keeping pace with several new agents entering the clinical domain. These drugs are being studied as single agents and in combination with chemotherapy and hormonal therapy [30].

11.6 Poly(ADP-Ribose) Pathway

There are a total of 17 members of the poly (ADP-ribose) polymerase (PARP) family, of which PARP-1 and PARP-2 orchestrate repair of single-stranded breaks in DNA [32]. These bind to DNA where there is damage and then start repair by ribosylation of nearby proteins, leading to base-excision repair at the site of damage and downstream effects on transcription and differentiation. Blocking of PARPs through competitive inhibition of the catalytic domain results in accumulation of DNA damage and cell death. BRCA1 and BRCA2 are tumour suppressor genes which plays important in DNA repair at sites of double-stranded breaks. Homologous recombination at DNA-damaged sites is an error free method of DNA repair mediated by Rad51 which is dependent on normal BRCA function. Mutations of BRCA genes drive the cellular machinery to rely on higher error prone methods of DNA repair and in turn promote genomic instability. The primitive studies of PARP inhibitors in BRCA-deficient tumours noted that, although mutations in BRCA increased tumour sensitivity to certain cytotoxic therapies, PARP inhibition causes cell death in this population which is approximately three-fold over conventional treatment. By letting single-stranded breaks unchecked by PARP inhibition, double-stranded DNA breaks are increased in cells that already lack DNA repair capability, a process which is known as synthetic lethality. Normal cells who have intact BRCA function will repair their double-stranded DNA breaks, making these tumour cells more prone to this treatment as compared to normal tissue.

PARP inhibitors are now being used in patients with BRCA-positive ovarian cancer. Among

patients with BRCA mutations and ovarian carcinoma treated with olaparib, a response rate of 41–53% was noted [33]. Side effects of olaparib include secondary myeloid leukemias, GI complaints, fatigue, and myelosuppression. The activity of PARP inhibitors may not be limited to patients with germline BRCA mutations. Approximately 50% of undifferentiated and high-grade serous ovarian cancers have a loss of BRCA1 function [34]. Some tumours have BRCA-like functional losses by inactivation of BRCA genes or defects in other genes required for BRCA-associated DNA repair that give in a clinical outcome which is similar to cancers with BRCA mutations. Many evidences highlight that PARP inhibitors results in increased cytotoxic effects of chemotherapy and radiation without regard to BRCA function. These substitute mechanisms of propagating cytotoxic DNA damage has resulted in expansion of the usage of PARP inhibitors in substantial number of malignancies. PARP inhibitors are currently being tested alone and in combination with chemotherapeutic agents, which may induce a susceptible tumour homologous recombination phenotype, to evaluate the potential risks and benefits of these drugs among patients who have impaired and normal BRCA function.

11.7 Principles of Immunotherapy

11.7.1 Tumour Immunbiology and Immunotherapy

As stated by Rushdan Noor, Eng Hseon Tay, and Jeffrey Low in the handbook of gynaecologic oncology "it is an important aspect of cancer biology is the tumour microenvironment, which contributes to tumour initiation, tumour progression and responses to therapy. Cells and molecules of the immune system are basic parts of the tumour microenvironment. Tumour cells express majority of the same cell surface antigens (e.g. HLA antigen) as seen in normal cells. Many tumour cells express specific antigens that are not found in similar normal cells." These are termed as tumour-associated antigens (TAAs). See Fig. 3.

Recognition of antigen by T-cells involves binding of the T-cell receptor to specific major histocompatibility complex (MHC)-peptide combination. By studying the T-cells that identify tumours, many TAAs have now been discovered. Tumour-associated antigens can be classified into five broad groups: (1) mutated antigens (e.g. mutated beta-catenin, caspase, k-ras, in CML, (2) cancer-testis antigens (restricted to testis germ cells, ovary and trophoblast, e.g. MAGE, BAGE, etc.), (3) overexpressed antigen (can be found in normal cell, e.g. p53, Her2/neu), (4) differentiation antigens (only expressed on particular tissue types, e.g. prostate specific antigen, tyrosinase, etc.), (5) oncogenic viral products (in virally induced cancers, e.g. Epstein–Barr virus antigens in lymphomas, HPV virus antigens E6, E7 in anogenital cancers, etc.).

Arguable evidences which suggest humans have tumour limiting factors (immunosurveillance):

1. Spontaneous regression has been reported rarely in cancers such as melanoma, renal cancer, and neuroblastoma. Many have observed that this tumour regression was preceded by surgical intervention (e.g. biopsy, partial resection), infections, administration of bacterial vaccines, transfusion reaction, etc.).
2. Self-healing melanomas.
3. Regression of metastases after resection of primary neoplasms.
4. Regression of tumour after non-cytotoxic doses of chemotherapy.
5. Reappearance of metastases after a long latent period.
6. Frequent failure of circulating tumour cells to form metastases.
7. Infiltration of tumours by mononuclear cells.
8. Higher incidence of tumours after clinical immunosuppression.
9. High incidence of tumour in immune deficiency diseases.
10. Increased incidence of malignancy with aging.

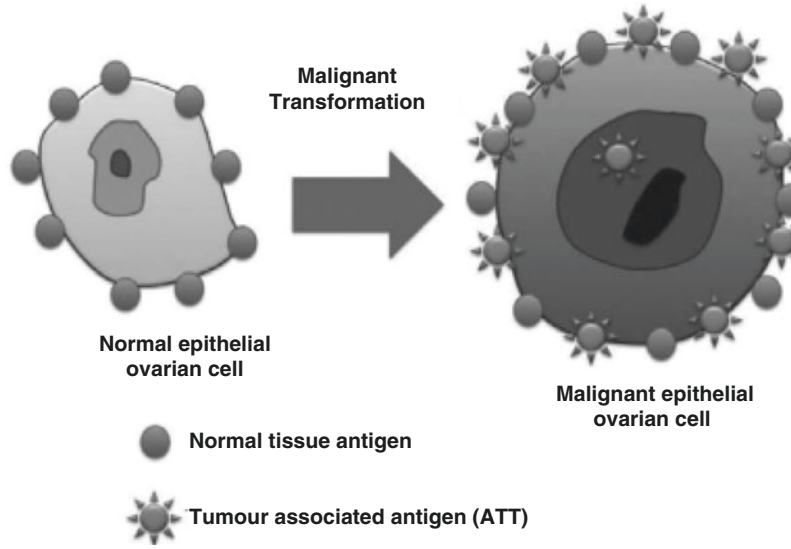

Fig. 3 Tumor-associated antigens are additionally expressed on the tumor cell surface. (Adapted with Permission from 2014 Pan Stanford Publishing Pte. Ltd through email communication)

The first report of successful immunotherapy was in 1881 by William Coley, who treat sarcomas by administrating bacterial toxins [35]. The ultimate goal of immunotherapy is the complete annihilation of all neoplastic cells. Effectiveness of immunotherapy is seen in neoplasms that are highly antigenic, such as Burkitt's lymphoma, malignant melanoma, and neuroblastoma. Tumour can change their antigenic profile and diminish the immune response. Some tumours can down-regulate many of the molecules that are involved in the processing and presentation of the peptide on MHC class I, and that changes occur in the antigenic profile of tumours as they progress and metastasize. The tumour microenvironment has also immunosuppressive properties because of hypoxia, induction, and recruitment of suppressor cells, oxidative stress, etc. This is why many immunotherapies have failed. They may be combined with radiotherapy or chemotherapy. Cancer vaccine has also shown quite a promise in immunotherapy. However, there are three main limitations: firstly, to identify the "correct antigen" that is dissimilar from a normal cell to minimize self-destruction, secondly to find the right adjuvant to enhance an immune response [at present only two adjuvants, aluminum-based salt and squalene oil-water–emulsion (MF56)]; other potential adjuvants are cytokines, bacterial products, heat shock protein, viral-like particles, etc., and finally to induce the right immune response, which is efficient in eradicating tumour cells, sustainable and excellent immune memory. The eventual goal of vaccine-based cancer immunotherapy is to obtain a robust immune response which will result in the eradication of the tumour as well as generate a long-term memory response in order to keep cancer in check.

12 Clinical Practice in Gynaecologic Oncology

12.1 Suitability for Immunotherapy

The outcome for patients can be improved by making primary therapy more effective or by exploring the application of "consolidation" or "maintenance" approaches to patients in a complete primary or subsequent remission. One important issue in evaluating immunotherapeutic approaches in ovarian cancer is to decide where the novel agent should be evaluated in the disease course. In general, the minimal disease state is sought, and the remission populations are best suited. The utility of additional treatment in patients who are in clinical complete remission was first recognized in acute leukemia, and additional "consolidation" or "mainte-

nance" chemotherapy significantly enhanced the outcome for some of these patients. However, these concepts have not shown similar results in solid tumour therapy, and hence the nomenclature remains puzzling. Consolidation therapy is best applied to those strategies that are of limited duration, such as a fixed immunization course, and "maintenance" is best used to describe interventions that continue for years (or until progression) such as with trastuzumab. In ovarian cancer, there were no statistically significant improvement in overall survival, which was seen in randomized consolidation study. Negative randomized consolidation approaches include both subcutaneous and intraperitoneal interferon-α, high-dose chemotherapy, continued intravenous carboplatin versus whole abdominal radiotherapy (WART), chemotherapy versus observation versus WART, intraperitoneal radioactive phosphorus (phosphorus 32), "non-cross-resistant" chemotherapy in the form of cisplatin and 5-fluorouracil for three cycles or topotecan for four cycles, the monoclonal antibody oregovomab, which targets CA-125, and the SMART study [36–38]. Consolidation strategies have generally been used in the first remission population; investigational strategies in the second and third remission groups have been rare and all likewise negative to date [39]. Patients with ovarian cancer in remission are ideal candidates for an immunotherapeutic strategy. Recent data highlight the homogeneity of the second and third remission groups who have a progression-free survival (PFS) interval of fewer than 12 months so that hints of efficacy from a given immunotherapeutic approach could be recognized with a shorter follow-up interval than that required in the first remission. The number of therapeutic strategies under investigation for immunotherapy in patients with ovarian cancer is large. Most trials are pilot studies or phase 1 trials that has assessed safety and immunogenicity. Some of them have shown improved outcomes with surrogates such as an antibody or T-cell response, and most current trials aim to produce cellular responses. The number of adequately powered randomized trials is few, however, and none has shown definitive efficacy to date.

12.2 Antibodies Used as Immunogens

Although some antibodies are administered in the treatment of patients with cancer to convey passive immunity, they may also be used as immunogens and can elicit a complex immune response. Oregovomab (MAb B43.13), which is an IgG1k subclass murine monoclonal antibody that binds with high affinity (1.16×10^{10}/M) to circulating CA-125, has been evaluated. Both cellular and humoral immune responses have been seen with the production of anti-oregovomab antibodies (Ab2), T-helper cells, and cytotoxic T-cells in addition to the human anti-mouse antibody (HAMA) response. Nonrandomized studies have consistently shown longer overall survival with immune response. A randomized placebo-controlled trial in patients with stage III or IV epithelial ovarian cancer in first clinical remission receiving oregovomab or placebo showed no benefit using the intent-to-treat population. However, a favorable subgroup of patients showed a time to progression advantage favoring vaccination of 24 months versus 10.8 months (hazard ratio, 0.543; 95% CI, 0.287–1.025). This subgroup was appropriately considered to be hypothesis-generating, and a follow-up study enrolled 354 patients using the characteristics of this group as eligibility criteria. The median time to progression was 10.3 months (95% CI, 9.7–13.0 months) for the oregovomab group and 12.9 months (95% CI, 10.1–17.4 months) for the placebo group, showing no benefit to oregovomab immunotherapy [38]. Another antibody strategy is immunization with an anti-idiotype vaccine. The hypothesis is that the antigenicity of the immunogen can be increased by presenting the desired epitope to the now tolerant host in a different molecular environment. The "immune network hypothesis," which provided the foundation for this approach, was initially proposed in the early 1970s and explains an interconnected group of idiotypes that are expressed by antibod-

ies. The proposed mechanism assumes that immunization with a given antigen will generate the production of antibodies against this antigen (termed Ab1). Ab1 can generate anti-idiotype antibodies against Ab1, classified as Ab2. Some of the anti-idiotypic antibodies (Ab2β) express the internal image of the antigen recognized by the Ab1 antibody and can be used as surrogate antigens. Immunization with Ab2β (the anti-idiotype antibody) can cause the production of anti-anti-idiotype antibodies (classified as Ab3) that recognize the corresponding original antigen identified by Ab1. Ab3 antibodies are also denoted Ab1′ to show that they may differ in their other epitopes compared with Ab1. A previous phase 1/2 study of abagovomab, the anti-idiotype monoclonal antibody whose epitope mirrors CA-125, suggested that Ab3 production was associated with overall survival. Other studies have shown an increase in interferon-γ expression of CA-125-specific CD8 + T-cells following immunization, but there has been no specific correlation between the induction of Ab3 and frequencies of CA-125-specific cytotoxic T-lymphocytes and T-helper cells. The efficacy of abagovomab in patients in the first remission was evaluated in an international phase 3, randomized, double-blind, placebo-controlled study ongoing in approximately 120 study locations (MIMOSA Trial). Outcomes were recurrence-free survival, overall survival, and safety. Preliminary blinded immunogenicity results were reported with 888 patients enrolled in the study and showed that 68% and 69% of all patients were positive for Ab3 (median values, 62,000 ng/mL and 337,000 ng/mL, respectively), whereas 53% and 63% of patients were positive for HAMA (median values, 510 ng/mL and 644 ng/mL, respectively).

12.3 Cancer-Testis Antigen Vaccines

Cancer-testis antigens represent a distinct class of differentiation antigens. The family has grown from the original melanoma-associated antigen 1 (MAGE-1) identified in a melanoma cell line to 100 cancer-testis genes or gene families identified in a recent database established by the Ludwig Institute for Cancer Research [40]. These antigens share several characteristics, including preferential expression in normal tissues on the testis and expression in tumours of varying histology (including ovarian cancer), and many are members of multigene families that are mostly encoded on chromosome X. Cancer-testis antigen expression has been correlated with clinical and pathologic parameters in a variety of tumours. MAGE-A4 expression shows an inverse correlation between expression and patient survival, for example, in ovarian cancer. The NY-ESO-1 antigen, initially defined by SEREX in esophageal cancer, is expressed in several tumours, including 40% of epithelial ovarian cancers. NY-ESO-1 MHC class I and II-restricted epitopes (recognized by CD8 + cytotoxic and CD4 + helper T-cells) have been characterized, including those recognized in conjunction with human leukocyte antigen (HLA)-A2 as well as with other haplotypes. Both NY-ESO-1 peptides and full recombinant protein have been administered to patients on protocols with immunogenicity as the primary endpoint with various adjuvants. Vaccination has been shown to induce both humoral and T-cell responses [41]. In a phase 1 trial in patients with epithelial ovarian cancer in the first remission immunized with HLA-A*0201–restricted NY-ESO-1b peptide with montanide ISA-51 as the adjuvant [42], treatment was well tolerated. Seven (77%) of nine patients showed T-cell immunity by tetramer and ELISPOT analyses. Multiple approaches have been used to try and enhance the inherently limited immunogenicity of these peptide vaccinations. Some have included amino acid substitution at different anchor positions of Melan-A/MART-126-352L; terminal alteration of MART-127-35; replacement of cysteine residues for NY-ESO-1; modification of T-cell receptor-interacting amino acid residues for carcinoembryonic antigen; and loading of peptides onto autologous dendritic cells. In addition, cytokines and costimulatory molecules have been administered.

12.4 Dendritic Cell-Based Vaccines

Dendritic cells act as antigen-presenting cells. They endocytose, process, and then present tumour antigens to T-cells. Many strategies are currently underway to manipulate the dendritic cell for use in immunotherapy. Dendritic cells have been pulsed with tumour-associated peptides or proteins and mRNA-encoded receptors such as folate receptor-α [43]. Other vaccines have been developed by the viral transduction of dendritic cells with tumour-specific genes or through transfection with liposomal DNA or RNA. Another strategy that has been tried to avoid the need to specifically define the effective tumour-associated antigens is to pulse them with tumour lysates or tumour protein extracts. In many cases, preclinical models have suggested protective immunity to subsequent tumour challenge, which supports further interest in investigating the approach. A specific example includes a study by Czerniecki et al. [44] in which advanced breast and ovarian cancer patients were treated with dendritic cells pulsed with HER-2/neu or MUC-1–derived peptides. In 50% of patients, peptide-specific cytotoxic T-cell lymphocytes were generated. Side effects were minimal. Gong and colleagues [45] fused human ovarian cells to human dendritic cells and likewise showed the proliferation of autologous T-cells, including cytotoxic T-cell activity with lysis of autologous tumour cells by an MHC class I restricted mechanism (i.e., demonstrating that the effector cells had the desired activity). Heat shock proteins, which are molecular chaperones that facilitate protein folding, have also been isolated, along with accompanying peptides and used as immunogens. Heat shock peptide complexes have been shown to interact with dendritic cells via the CD91 receptor. The heat shock proteins are taken up by endocytosis, are cross-presented by MHC-I molecules on the dendritic cells, and result in activation of naïve CD8 + cells along with upregulation of costimulatory molecules and the production of cytokines. Many reported studies have similar immunologic endpoints, but the clinical interpretation is often difficult from phase 1/2 trials without comparators.

12.5 Vaccines Designed to Generate Antibody Responses

Most current vaccines seek to generate cellular responses (often with an accompanying humoral response), but a vaccine is currently in phase 2 randomized trial in ovarian cancer (Gynaecology Oncology Group [GOG] Study 255) that evaluates a vaccine approach primarily designed to augment antibodies. Techniques for the chemical and enzymatic synthesis of carbohydrate and glycopeptide antigens have allowed the development of a range of synthetic vaccines that depend on antibody production and ADCC as the primary effectors. A variety of options such as different adjuvant therapies, schedules, and methods of conjugation have been tried to enhance immunogenicity. A proposed optimal construct has consisted of an antigen (single or multiple) with the carrier protein keyhole limpet hemocyanin (KLH) and the saponin adjuvant QS-21 (or OPT-821) [46].

12.6 Adoptive Cellular Therapy

Using the adoptive cellular therapy approach, one selects and activates many lymphocytes and introduces them into a modified host environment with a selected target. One way T-cells may be modified to recognize tumour-associated antigens is to introduce ex vivo a gene encoding artificial T-cell receptors termed chimeric antigen receptors (CARs) against a specific tumour-associated antigen. The first phase 1 study in patients with epithelial ovarian cancer using gene-modified autologous T-cells with reactivity against ovarian cancer-associated antigen α-folate receptor (FR) has been reported [47]. Cohort 1 received T-cells with IL-2 and cohort 2 received dual specific T-cells followed by allogeneic peripheral blood mononuclear cells. There was no reduction which was seen in tumour burden of any patient. Polymerase chain reaction examination showed that gene-modified T-cells were present in the circulation 2 days after trans-

fer but thereafter declined. An inhibitory factor was developed in the serum of 3 out of 6 patients tested over the treatment period significantly reduced the ability of gene-modified T-cells to respond against FR-positive tumour cells. Further studies are needed to use strategies to increase T-cell persistence. The chimeric receptor approach continues to evolve in specificity against targets expressed in ovarian cancer such as the LeY carbohydrate antigen (expressed on 70% of ovarian cancer cells) or HER-2/neu. Most recently, receptors have been engineered to target the extracellular domain (termed MUC-CD) of MUC16 (CA125), which is expressed in most ovarian carcinoma [48]. In vitro, these CAR-modified, MUC-CD-targeted T-cells showed MUC-CD-specific cytolytic activity against ovarian cell lines, and infusion into severe combined immunodeficiency (SCID)-beige mice bearing orthotopic human MUC-CD–positive ovarian carcinoma tumours showed delayed disease progression or eradication. Clinical trials are planned. One necessary challenge to overcome is how to circumvent the multiple mechanisms in the tumour microenvironment that inhibit tumour-targeted T-cells. Options under investigation include administering T-cells after lymphodepleting chemotherapy, antibody-based blockade of inhibitory ligands, and infusion of proinflammatory cytokines such as IL-12.

12.7 Whole Tumour Antigen Vaccines

This strategy seeks to overcome some of the potential problems associated with trying to generate specific immune responses. In the latter case, the response may simply miss the target, it can be limited to only the epitopes provided on the stimulating antigen and drive variants of tumour cells that can evade the immune response (immunoediting), or it may be restricted to small numbers of patients of a certain HLA type, as in the case of using HLA-restricted peptides [42]. The reason for using whole tumour antigen vaccines is that it allows one to immunize without needing to define the tumour-associated antigens. They can be derived from autologous tumour cells or using an allogeneic strategy. One obvious challenge in using whole tumour antigen vaccines is that the tumour is currently residing in a host where tolerance to the tumour is already present. This tolerance is likely produced in multiple ways, including the production of IL-10 and transforming growth factor (TGF)-β to inhibit T-cell and dendritic cell functions, VEGF to inhibit dendritic cell maturation and differentiation, and soluble Fas ligand, which induces lymphocyte apoptosis. The whole tumour immunogen, therefore, is processed or modified in some way in an attempt to overcome this. Strategies have included using apoptotic whole tumour cells (developed with a lethal dose of irradiation), using necrotic tumour cell lysates (created with repetitive freezing and thawing and often administered as pulsed dendritic cells), and constructing dendritic cell/tumour fusion vaccines [49]. The issue of how to increase the immunogenicity of whole tumour vaccines remains a priority. One effective approach has been the use of a replication-deficient herpes simplex virus to infect tumour cells, which are subsequently engulfed and show enhanced ability to both activate NK cells and provide a costimulatory signal for T-cells.

12.8 Immune Checkpoint Inhibitors

Immune checkpoint synapses consist of several co-inhibitory molecules that are primarily responsible for limiting T-cell receptor signaling and abrogating immune responses. This strategic process set in place by the immune system is useful to halt immune responses in individuals after microbial infections are resolved, or in the development of self-tolerance to limit autoimmune disease. However, in cancer, high levels of immune checkpoint molecules on immune cells or on tumour cells are

often associated with exhausted T-cells, which are incapable of developing aggressive anti-tumour responses, as well as with resistance to several classes of therapy [50–52]. .Immune checkpoints (IC) present potent immune-suppressive mechanisms in cancer, and blocking of two of these pathways in particular has provided useful therapeutic alternatives to improve survival in many cancer types. Briefly, the binding of CD28 on T-cells to B7-1/B7-2 (CD80/CD86) on antigen-presenting cells (APC) results in costimulatory anti-tumour responses. However, co-inhibitory molecule CTLA-4 on T-cells has a higher affinity for B7-1/B7-2 molecules than does CD28, and the preferential binding of CTLA-4 to B7-1/B7-2 blocks IL-2 release from T-cells and limits T-cell proliferation.

12.9 Immune Checkpoint Blockade Therapy in Endometrial Cancer

Immune checkpoint inhibitors have shown efficacy in multiple advanced solid tumours, predominantly among MMRd and MSI-H cancers and those with a high tumour mutational burden, such as endometrial cancer. The phase II KEYNOTE-158 study evaluated the anti-tumour activity and safety of pembrolizumab in previously treated, advanced non-colorectal MSI-H/MMRd cancers. Patients were treated with a fixed dose of pembrolizumab 200 mg IV once every 3 weeks for 2 years or until disease progression, unacceptable toxicity, or patient withdrawal. Among patients with a broad range of solid tumours including 27 tumour types, there were 49 patients with endometrial cancer (21% of the treatment population). In the cohort of patients with endometrial cancer, the ORR was 57.1%, with eight patients (16%) achieving a complete response and 20 patients (41%) achieving a partial response. The median PFS was 25.7 months. In the entire study cohort of 233 patients, 64.8% of patients had treatment-related adverse events and 14.6% had grade 3–5 treatment-related adverse events, with one grade 5 event related to pneumonia. The most common treatment-related adverse events were fatigue, pruritus, diarrhea, and asthenia. This study further indicated that MSI/MMRd status could be a predictor of the response to PD-1 blockade in endometrial cancer [53]. KEYNOTE-146/Study 111 was a single-arm, open label, phase Ib/II study to evaluate the safety and efficacy of lenvatinib plus pembrolizumab in advanced solid tumours, including endometrial carcinoma. Patients received lenvatinib 20 mg once daily orally plus pembrolizumab 200 mg IV once every 3 weeks, based on the recommended dosing from the phase Ib portion of the study. The final primary efficacy analysis was reported for the patient cohort with advanced endometrial carcinoma. The primary endpoint was ORR at 24 weeks (ORRWk24). The ORRWk24 was 38% in the cohort of 108 patients who were previously treated with conventional therapy. For 94 patients with MSS/MMRp tumours, ORR as measured by immune-related RECIST (irRECIST) was 37.2% versus 63.6% for 11 patients with MSI-H/MMRd tumours [54].

12.10 Immune Checkpoint Blockade Therapy in Cervical Cancer

Conventional treatment options for metastatic/recurrent cervical cancer additionally includes radiotherapy and chemotherapy, and this treatment is most often not sufficiently effective for disease management at this late stage. In a phase II KEYNOTE-158 trial, pembrolizumab was investigated in a single cohort trial of 98 patients with recurrent/metastatic cervical cancer. Of 77 patients, the ORR was 14.3% (95% CI: 7.4, 24.1), with 11.7% partial responses and 2.6% complete responses, whereas no responses were found in patients with tumours

not expressing PD-L1 (NCT02628067). With this outcome, pembrolizumab was subsequently approved in 2018 for recurrent/metastatic cervical cancer patients with PD-L1 positive tumours [53].

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
2. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2010;141:1117–34.
3. Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability: an evolving hallmark of cancer. Nat Rev Mol Cell Biol. 2010;11:220–8.
4. Skipper HE. Kinetics of mammary tumor cell growth and implications for therapy. Cancer. 1971;28:1479–99.
5. Norton LA. A Gompertzian model of human breast cancer growth. Cancer Res. 1988;48:7067–71.
6. Raguz S, Yague E. Resistance to chemotherapy: new treatments and novel insights into an old problem. Br J Cancer. 2008;99:387–91.
7. Gerlinger M, Swanton C. How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine. Br J Cancer. 2010;103:1139–43.
8. Goldie JH, Coldman AJ. A mathematical model for relating the drug sensitivity of tumors to the spontaneous mutation rate. Cancer Treat Rep. 1979;63:1727–33.
9. Bookman MA, Brady MF, McGuire WP, et al. Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a phase III trial of the Gynecologic Cancer InterGroup. J Clin Oncol. 2009;27:1419–25.
10. Tan DS-W, Gerlinger M, The B-T, et al. Anti-cancer drug resistance: understanding the mechanisms through the use of integrative genomics and functional RNA interference. Eur J Cancer. 2010;46:2166–77.
11. Sakai S, Swisher EM, Karlan BY, et al. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature. 2008;451:1116–21.
12. Cress RD, O'Malley CD, Leiserowitz GS, et al. Patterns of chemotherapy use for women with ovarian cancer: a population based study. J Clin Oncol. 2003;21:1530–5.
13. Citron ML, Berry DA, Cirrincione C, et al. Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741. J Clin Oncol. 2003;21:1431–9.
14. Pfisterer J, Plante M, Vergote I, et al. Gemcitabine plus carboplatin compared with carboplatin in patients with platinum-sensitive recurrent ovarian cancer: an intergroup trial of the AGO-OVAR, the NCIC CTG, and the EORTC GCG. J Clin Oncol. 2006;24:2699–707.
15. Siemann DW, Horsman MR. Modulation of the tumor vasculature and oxygenation to improve therapy. Pharmacol Ther. 2015;153:107–24.
16. Borner M, Scheithauer W, Twelves C, et al. Answering patients' needs: oral alternatives to intravenous therapy. Oncologist. 2001;4:12.
17. Dedrick RL, Myers CE, Bungay PM, et al. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat Rep. 1978;62:1–9.
18. Markman M. Intraperitoneal therapy of ovarian cancer. Semin Oncol. 1998;25:356–60.
19. Markman M, Kelsen D. Efficacy of cisplatin-based intraperitoneal chemotherapy as treatment of malignant peritoneal mesothelioma. J Cancer Res ClinOncol. 1992;118:547–50.
20. Rocha-Lima CM, Soares HP, Raez LE, Singal R. EGFR targeting of solid tumors. Cancer Control. 2007;14(3):295–304.
21. Wiezorek J, Holland P, Graves J. Death receptor agonists as a targeted therapy for cancer. Clin Cancer Res. 2010;16(6):1701–8.
22. Alberts DS, Marth C, Alvarez RD, et al. Randomized phase 3 trial of interferon gamma-1b plus standard carboplatin/paclitaxel versus carboplatin/paclitaxel alone for first-line treatment of advanced ovarian and primary peritoneal carcinomas: results from a prospectively designed analysis of progression-free survival. Gynecol Oncol. 2008;109(2):174–81.
23. Giuntoli RL II, Webb TJ, Zoso A, et al. Ovarian cancer-associated ascites demonstrates altered immune environment: implications for antitumor immunity. Anticancer Res. 2009;29(8):2875–84.
24. Burger RA, Sill MW, Monk BJ, Greer BE, Sorosky JI. Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a Gynecologic Oncology Group study. J Clin Oncol. 2007;25:5165–71.
25. Cannistra SA, Matulonis UA, Penson RT, et al. Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer. J Clin Oncol. 2007;25(33):5180–6.
26. Garcia AA, Hirte H, Fleming G, et al. Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the California, Chicago, and Princess Margaret Hospital phase II consortia. J Clin Oncol. 2008;26(1):76–82.
27. Aghajanian C, Sill MW, Darcy KM, et al. A phase II evaluation of bevacizumab in the treatment of recurrent or persistent endometrial cancer: a Gynecologic Oncology Group (GOG) study. J Clin Oncol. 2009;27(15_Suppl):5531.
28. Monk BJ, Sill MW, Burger RA, Gray HJ, Buekers TE, Roman LD. Phase II trial of bevacizumab in the treatment of persistent or recurrent squamous cell car-

cinoma of the cervix: a Gynecologic Oncology Group study. J Clin Oncol. 2009;27(7):1069–74.
29. Faried LS, Faried A, Kanuma T, et al. Expression of an activated mammalian target of rapamycin in adenocarcinoma of the cervix: a potential biomarker and molecular target therapy. Mol Carcinog. 2008;47(6):446–57.
30. Bansal N, Yendluri V, Wenham RM. The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies. Cancer Control. 2009;16(1):8–13.
31. Slomovitz BM, Lu KH, Johnston T, et al. A phase 2 study of the oral mammalian target of rapamycin inhibitor, everolimus, in patients with recurrent endometrial carcinoma. Cancer. 2010;116(23):5415–9.
32. Miwa M, Masutani M. PolyADP-ribosylation and cancer. Cancer Sci. 2007;98(10):1528–35.
33. Fong PC, Boss DS, Yap TA, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009;361(2):123–34.
34. Press JZ, De Luca A, Boyd N, et al. Ovarian carcinomas with genetic and epigenetic BRCA1 loss have distinct molecular abnormalities. BMC Cancer. 2008;8:17.
35. Coley WB. The treatment of malignant tumours by repeated inoculations of erysipelas: with a report of ten original cases. Am J Med Sci. 1893;105:487.
36. Alberts DS, Hannigan EV, Liu PY, et al. Randomized trial of adjuvant intraperitoneal alpha-interferon in stage III ovarian cancer patients who have no evidence of disease after primary surgery and chemotherapy: an intergroup study. Gynecol Oncol. 2006;100(1):133–8.
37. Verheijen RH, Massuger LF, Benigno BB, et al. Phase III trial of intraperitoneal therapy with yttrium90-labeled HMFG1 murine monoclonal antibody in patients with epithelial ovarian cancer after a surgically defined complete remission. J Clin Oncol. 2006;24(4):571–8.
38. Berek J, Taylor P, McGuire W, Smith LM, Schultes B, Nicodemus CF. Oregovomab maintenance monoimmunotherapy does not improve outcomes in advanced ovarian cancer. J Clin Oncol. 2009;27(3):418–25.
39. Sabbatini P. Consolidation therapy in ovarian cancer: a clinical update. Int J Gynecol Cancer. 2009;19(Suppl 2):S35–9.
40. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science. 1991;254:1643–7.
41. Odunsi K, Qian F, Matsuzaki J, et al. Vaccination with an NY-ESO-1 peptide of HLA class I/II specificities induces integrated humoral and T cell responses in ovarian cancer. Proc Natl Acad Sci U S A. 2007;104(31):12837–42.
42. Diefenbach CS, Gnjatic S, Sabbatini P, et al. Safety and immunogenicity study of NY-ESO-1b peptide and montanide ISA-51 vaccination of patients with epithelial ovarian cancer in high-risk first remission. Clin Cancer Res. 2008;14(9):2740–8.
43. Hernando JJ, Park TW, Fischer HP, et al. Vaccination with dendritic cells transfected with mRNA encoded folate-receptor-alpha for relapsed metastatic ovarian cancer. Lancet Oncol. 2007;8(5):451–4.
44. Czerniecki BJ, Koski GK, Koldovsky U, et al. Targeting HER-2/neu in early breast cancer development using dendritic cells with staged interleukin-12 burst secretion. Cancer Res. 2007;67(4):1842–52.
45. Gong J, Apostolopoulos V, Chen D, et al. Selection and characterization of MUC1-specific $CD8^+$ T cells from MUC1 transgenic mice immunized with dendritic-carcinoma fusion cells. J Immunol. 2000;101(3):316–24.
46. Sabbatini PJ, Ragupathi G, Hood C, et al. Pilot study of a heptavalent vaccine-keyhole limpet hemocyanin conjugate plus QS21 in patients with epithelial ovarian, fallopian tube, or peritoneal cancer. Clin Cancer Res. 2007;13(14):4170–7.
47. Kershaw MH, Westwood JA, Parker LL, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res. 2006;12(20 Pt 1):6106–15.
48. Chekmasova AA, Brentjens RJ. Adoptive T cell immunotherapy strategies for the treatment of patients with ovarian cancer. Discov Med. 2010;9(44):62–70.
49. Hatfield P, Merrick AE, West E, et al. Optimization of dendritic cell loading with tumor cell lysates for cancer immunotherapy. J Immunother. 2008;31(7):620–32.
50. Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. Immunity. 2018;48:434–52.
51. Zhou G, Sprengers D, Boor PPC, Doukas M, Schutz H, Mancham S, Pedroza-Gonzalez A, Polak WG, de Jonge J, Gaspersz M, et al. Antibodies against immune checkpoint molecules restore functions of tumor-infiltrating T cells in hepatocellular carcinomas. Gastroenterology. 2017;153:1107–19.
52. Salmaninejad A, Valilou SF, Shabgah AG, Aslani S, Alimardani M, Pasdar A, Sahebkar A. PD-1/PD-L1 pathway: basic biology and role in cancer immunotherapy. J Cell Physiol. 2019;234:16824–37.
53. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, Geva R, Gottfried M, Penel N, Hansen AR, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase II KEYNOTE-158 study. J Clin Oncol. 2020;38:1–10.
54. Makker V, Taylor MH, Aghajanian C, Oaknin A, Mier J, Cohn AL, Romeo M, Bratos R, Brose MS, DiSimone C, et al. Lenvatinib plus pembrolizumab in patients with advanced endometrial cancer. J Clin Oncol. 2020;38:2981–92.