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Mechanisms of anticancer drugs

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SEARCH STRATEGY

The data in this chapter are supported by a Medline search using the key words chemotherapy and head and neck neoplasms, and focus on mechanisms of action of current and experimental drugs.

INTRODUCTION

The discovery of the toxic action of nitrogen mustards on cells of the haematopoietic system more than 50 years ago initially triggered research into the development of cytotoxic agents. The initial promise of these drugs in the management of haematological and other rare malignancies has not been sustained and cure of the more common epithelial malignancies when metastatic, remains an elusive goal.

Many of the current chemotherapeutic agents have been discovered as a result of screening compounds for cytotoxic potency *in vitro* against murine and/or human cancer cells or *in vivo* against rodent tumour models. With our better understanding of the molecular basis of cancer there is now interest in target-directed drug therapies. The aim being to develop agents that can modulate or inhibit specific molecular targets identified as being essential for tumour growth.

PRINCIPLES OF CHEMOTHERAPY

Many forms of chemotherapy are targeted at the process of cell division. The rationale being that cancer cells are more likely to be replicating than normal cells. Unfortunately as their action is not specific, they are associated with significant toxicity. An understanding of the principles of tumour biology and cellular kinetics is helpful to appreciate the mechanisms of action of cancer chemotherapy.

PRINCIPLES OF TUMOUR BIOLOGY

Cellular kinetics

CELL CYCLE

Uncontrolled cell division is a result of interference in the normal balance of the cell cycle. The cell cycle is divided into a number of phases governed by an elaborate set of

molecular switches (**Figure 4.1**). Normal nondividing cells are in G0. When actively recruited into the cell cycle they then pass through four phases:

1. **G1**: the growth phase in which the cell increases in size and prepares to copy its DNA;
2. **S (synthesis)**: which allows doubling of the chromosomal material;
3. **G2**: a further growth phase before cell division;
4. **M (mitosis)**: where the chromosomes separate and the cell divides.

At the end of a cycle the daughter cells can either continue through the cycle, leave and enter the resting phase (G0) or become terminally differentiated.
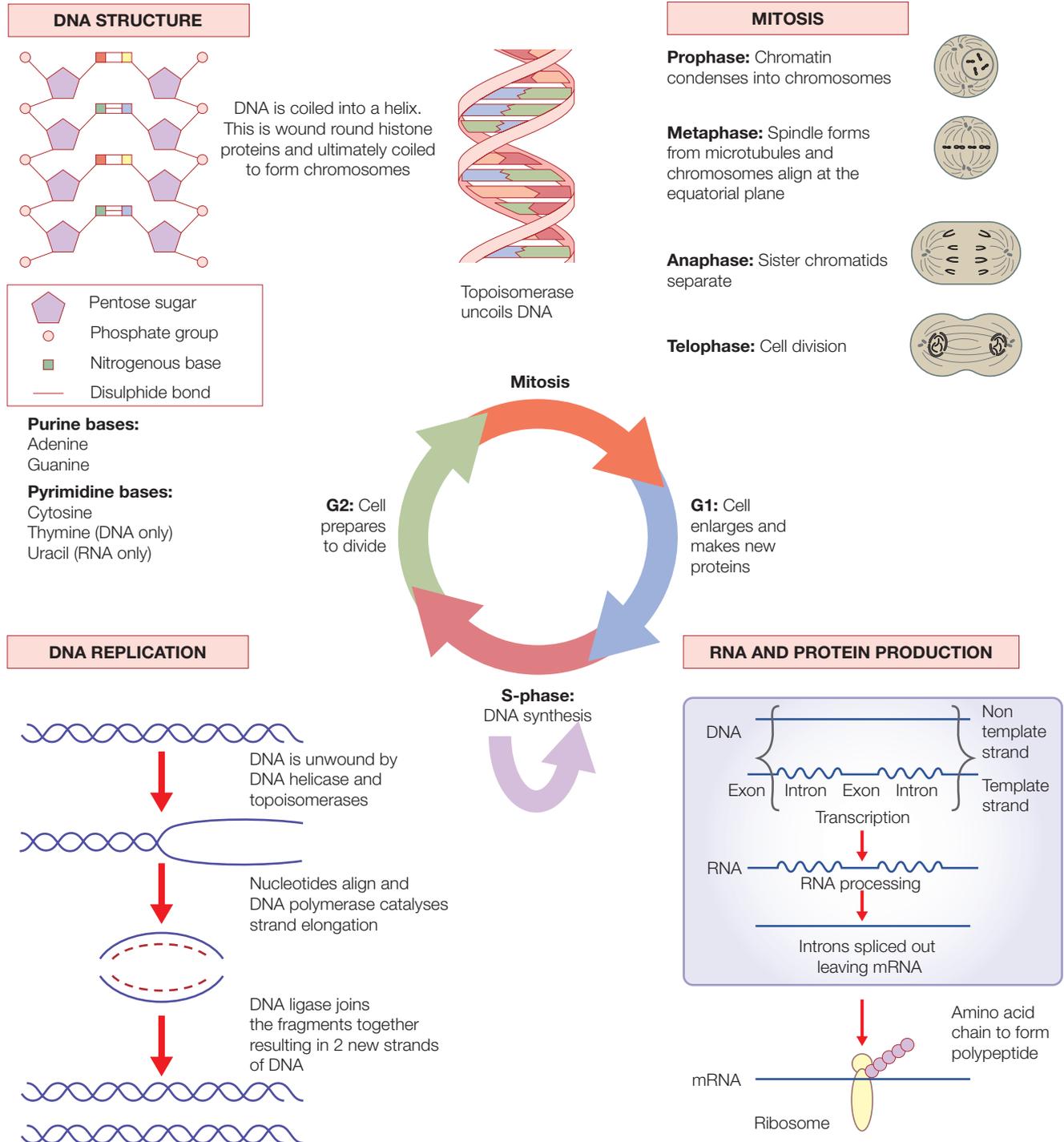
DNA STRUCTURE

DNA is coiled into a helix. This is wound round histone proteins and ultimately coiled to form chromosomes

Topoisomerase uncoils DNA

- ⬠ Pentose sugar
- ○ Phosphate group
- ▢ Nitrogenous base
- — Disulphide bond

Purine bases:
Adenine
Guanine

Pyrimidine bases:
Cytosine
Thymine (DNA only)
Uracil (RNA only)

MITOSIS

Prophase: Chromatin condenses into chromosomes

Metaphase: Spindle forms from microtubules and chromosomes align at the equatorial plane

Anaphase: Sister chromatids separate

Telophase: Cell division

Mitosis

G2: Cell prepares to divide

G1: Cell enlarges and makes new proteins

S-phase: DNA synthesis

DNA REPLICATION

DNA is unwound by DNA helicase and topoisomerases

Nucleotides align and DNA polymerase catalyses strand elongation

DNA ligase joins the fragments together resulting in 2 new strands of DNA

RNA AND PROTEIN PRODUCTION

DNA

Non template strand

Exon   Intron   Exon   Intron

Template strand

Transcription

RNA

RNA processing

Introns spliced out leaving mRNA

Amino acid chain to form polypeptide

mRNA

Ribosome

Figure 4.1   Cell cycle: possible targets for chemotherapy.

TUMOUR GROWTH

The kinetics of any population of tumour cells is regulated by the following:

- **doubling time**: the cell cycle time, which varies considerably between tissue types;
- **growth fraction**: the percentage of cells passing through the cell cycle at a given point in time which is greatest in the early stages;
- **cell loss**: which can result from unsuccessful division, death, desquamation, metastasis and migration.

Tumours characteristically follow a sigmoid-shaped growth curve, in which tumour doubling size varies with tumour size. Tumours grow most rapidly at small volumes. As they become larger, growth is influenced by the rate of cell death and the availability of blood supply.

Cell signalling

Cells respond to their environment via external signals called growth factors. These interact with cell surface receptors that activate an internal signalling cascade. This ultimately acts at the DNA level through transcription factors that bind to the promoter regions of relevant genes, stimulating the cell cycle and influencing many important processes including cell division, migration, programmed cell death (apoptosis).

ONCOGENES

Protooncogenes are involved in controlling normal cell growth. Mutated forms, known as oncogenes, can lead to inappropriate stimulation of the cell cycle and excessive cell growth. Alternatively, malignancy can also arise secondary to abnormal activation of a normal gene. The consequences of gene activation associated with tumour growth include:

- excess growth factor production;
- alteration of growth factor receptor genes so that they are permanently switched on;
- alteration of the intracellular cascade stimulating proliferation.

TUMOUR SUPPRESSOR GENES

These act as a natural brake on cell growth. Usually both alleles need to be lost for their function to be affected. This can have several important effects, which include:

- impairment of the inhibitory signals influencing receptor genes or intracellular signalling;
- loss of the counter signals controlling protooncogene function;
- inhibition of apoptosis, often as a consequence of a mutation of p53, the protein associated with DNA repair.

Metastatic spread

A tumour is considered malignant when it has the capacity to spread beyond its original site and invade surrounding tissue. Normally cells are anchored to the extracellular matrix by cell adhesion molecules, including the integrins. Abnormalities of the factors maintaining tissue integrity will allow local invasion and ultimately metastases of the tumour cells.

Mechanism of cell death

There are two main types of cell death: apoptosis and necrosis. Necrotic cell death is caused by gross cell injury and results in the death of groups of cells within a tissue. Apoptosis is a regulated form of cell death that may be induced or is preprogrammed into the cell (e.g. during development) and is characterized by specific DNA changes and no accompanying inflammatory response. It can be triggered if mistakes in DNA replication are identified. Loss of this protective mechanism would allow mutant cells to continue to divide and grow, thereby conserving mutations in subsequent cell divisions.

Many cytotoxic anticancer drugs and radiotherapy act by inducing mutations in cancer cells which are not sufficient to cause cell death, but which can be recognized by the cell, triggering apoptosis.

FRACTIONAL CELL KILL HYPOTHESIS AND DRUG DOSING

Theoretically the administration of successive doses of chemotherapy will result in a fixed reduction in the number of cancer cells with each cycle.[1] A gap between cycles is necessary to allow normal tissue recover. Unfortunately, these first-order dynamics are not observed in clinical practice. Factors such as variation in tumour sensitivity and effective drug delivery with each course result in an unpredictable cell response.

Clinical responses to antitumour therapies are defined by arbitrary criteria that have been used as part of the evaluation process in assessing the potential utility of novel agents.

- **Tumour size**:
  - complete response is defined as the apparent disappearance of the tumour;
  - partial response represents a reduction of more than 50 percent;
  - progression is defined as an increase in tumour size by more than 25 percent;
  - stable disease is an intermediate between partial response and tumour progression.
- **Tumour products**:
  - biochemical or other tests can be used to assess response, including circulating tumour markers.

CLASSIFICATION OF CHEMOTHERAPEUTIC AGENTS

Classification according to phase–specific toxicity

Cytotoxic drugs can be classified according to whether they are more likely to target cells in a particular phase of their growth cycle. More crudely, they can also be divided into whether they are more toxic to cells that are actively dividing rather than cells in both the proliferating and resting phases.

PHASE–SPECIFIC CHEMOTHERAPY

These drugs, such as methotrexate and vinca alkaloids, kill proliferating cells only during a specific part or parts of the cell cycle. Antimetabolites, such as methotrexate, are more active against S-phase cells (inhibiting DNA synthesis) whereas vinca alkaloids are more M-phase specific (inhibiting spindle formation and alignment of chromosomes).

Attempts have been made to time drug administration in such a way that the cells are synchronized into a phase of the cell cycle that renders them especially sensitive to the cytotoxic agent. For example, vinblastine can arrest cells in mitosis. These synchronized cells enter the S-phase together and can be killed by a phase-specific agent, such as cytosine arabinoside. Most current drug schedules, however, have not been devised on the basis of cell kinetics.

CELL CYCLE–SPECIFIC CHEMOTHERAPY

Most chemotherapy agents are cell cycle-specific, meaning that they act predominantly on cells that are actively dividing. They have a dose-related plateau in their cell killing ability because only a subset of proliferating cells remain fully sensitive to drug-induced cytotoxicity at any one time. The way to increase cell kill is therefore to increase the duration of exposure rather than increasing the drug dose.

CELL CYCLE–NONSPECIFIC CHEMOTHERAPY

These drugs, for example alkylating agents and platinum derivatives, have an equal effect on tumour and normal cells whether they are in the proliferating or resting phase. They have a linear dose–response curve; that is, the greater the dose of the drug, the greater the fractional cell kill.

Classification according to mechanism

Classifying cytotoxic drugs according to their mechanism of action is the preferred system in use between clinicians.

ALKYLATING AGENTS

These highly reactive compounds produce their effects by covalently linking an alkyl group (R-CH2) to a chemical species in nucleic acids or proteins. The site at which the cross-links are formed and the number of cross-links formed is drug specific. Most alkylating agents are bipolar, i.e. they contain two groups capable of reacting with DNA. They can thus form bridges between a single strand or two separate strands of DNA, interfering with the action of the enzymes involved in DNA replication. The cell then either dies or is physically unable to divide or triggers apoptosis. The damage is most serious during the S-phase, as the cell has less time to remove the damaged fragments. Examples include:

- nitrogen mustards (e.g. melphalan and chlorambucil);
- oxazaphosphorenes (e.g. cyclophosphamide, ifosfamide);
- alkyl alkane sulphonates (busulphan);
- nitrosureas (e.g. carmustine (BCNU), lomustine (CCNU));
- tetrazines (e.g. dacarbazine, mitozolomide and temozolomide);
- aziridines (thiopeta, mitomycin C);
- procarbazine.

HEAVY METALS

Platinum agents

These include carboplatin, cisplatin and oxaliplatin. Cisplatin is an organic heavy metal complex. Chloride ions are lost from the molecule after it diffuses into a cell allowing the compound to cross-link with the DNA strands, mostly to guanine groups. This causes intra- and interstrand DNA cross-links, resulting in inhibition of DNA, RNA and protein synthesis.

Carboplatin has the same platinum moiety as cisplatin, but is bonded to an organic carboxylate group. This leads to increased water solubility and slower hydrolysis that has an influence on its toxicity profile. It is less nephrotoxic and neurotoxic, but causes more marked myelosuppression.

Oxaliplatin belongs to a new class of platinum agent. It contains a platinum atom complexed with oxalate and a bulky diaminocyclohexane (DACH) group. It forms reactive platinum complexes that are believed to inhibit DNA synthesis by forming interstrand and intra-strand cross-linking of DNA molecules. Oxaliplatin is not generally cross-resistant to cisplatin or carboplatin, possibly due to the DACH group.

ANTIMETABOLITES

Antimetabolites are compounds that bear a structural similarity to naturally occurring substances such as vitamins, nucleosides or amino acids. They compete with

the natural substrate for the active site on an essential enzyme or receptor. Some are incorporated directly into DNA or RNA. Most are phase-specific, acting during the S-phase of the cell cycle. Their efficacy is usually greater over a prolonged period of time, so they are usually given continuously. There are three main classes.

Folic acid antagonists

Methotrexate competitively inhibits dihydrofolate reductase, which is responsible for the formation of tetrahydrofolate from dihydrofolate. This is essential for the generation of a variety of coenzymes that are involved in the synthesis of purines, thymidylate, methionine and glycine. A critical influence on cell division also appears to be inhibition of the production of thymidine monophosphate, which is essential for DNA and RNA synthesis. The block in activity of dihydrofolate reductase can be bypassed by supplying an intermediary metabolite, most commonly folinic acid. This is converted to tetrahydrofolate that is required for thymidylate synthetase function (**Figure 4.2**).

Pyrimidine analogues

These drugs resemble pyrimidine molecules and work by either inhibiting the synthesis of nucleic acids (e.g. fluorouracil (**Figure 4.3**)), inhibiting enzymes involved in DNA synthesis (e.g. cytarabine, which inhibits DNA polymerase) or by becoming incorporated into DNA (e.g. gemcitabine), interfering with DNA synthesis and resulting in cell death.

Purine analogues

These are analogues of the natural purine bases and nucleotides. 6-Mercaptopurine (6MP) and thioguanine are derivatives of adenine and guanine, respectively. A sulphur group replaces the keto group on carbon-6 in these compounds. In many cases, the drugs require initial activation. They are then able to inhibit nucleotide biosynthesis by direct incorporation into DNA.

CYTOTOXIC ANTIBIOTICS

Most antitumour antibiotics have been produced from bacterial and fungal cultures (often *Streptomyces* species). They affect the function and synthesis of nucleic acids in different ways.

- **Anthracyclines** (e.g. doxorubicin, daunorubicin, epirubicin) intercalate with DNA and affect the topoioismerase II enzyme. This DNA gyrase splits the DNA helix and reconnects it to overcome the torsional forces that would interfere with replication. The anthracyclines stabilize the DNA tomoisomerase II complex and thus prevent reconnection of the strands.
- **Actinomycin D** intercalates between guanine and cytosine base pairs. This interferes with the transcription of DNA at high doses. At low doses DNA-directed RNA synthesis is blocked.
- **Bleomycin** consists of a mixture of glycopeptides that cause DNA fragmentation.
- **Mitomycin C** inhibits DNA synthesis by cross-linking DNA, acting like an alkylating agent.

SPINDLE POISONS

Vinca alkaloids

The two prominent agents in this group are vincristine and vinblastine that are extracted from the periwinkle
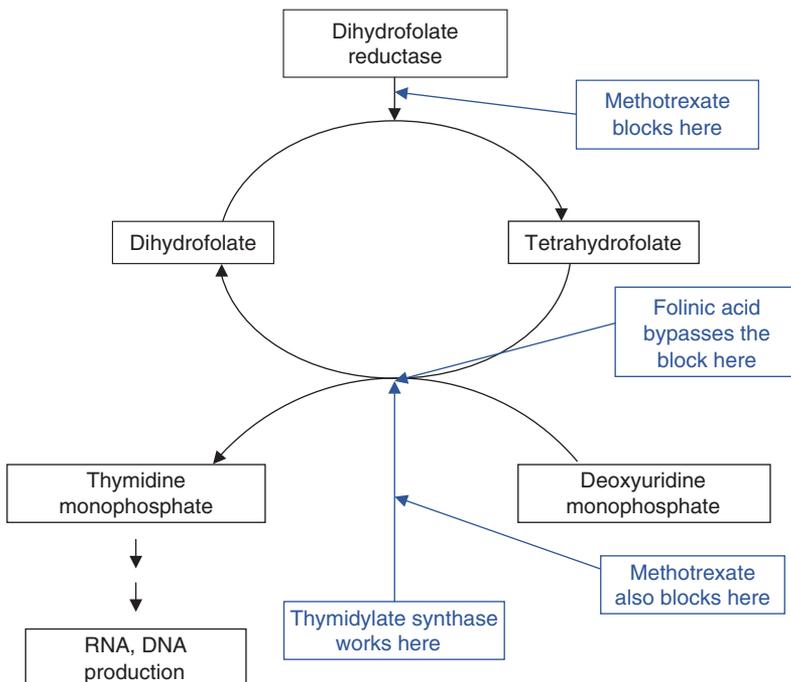

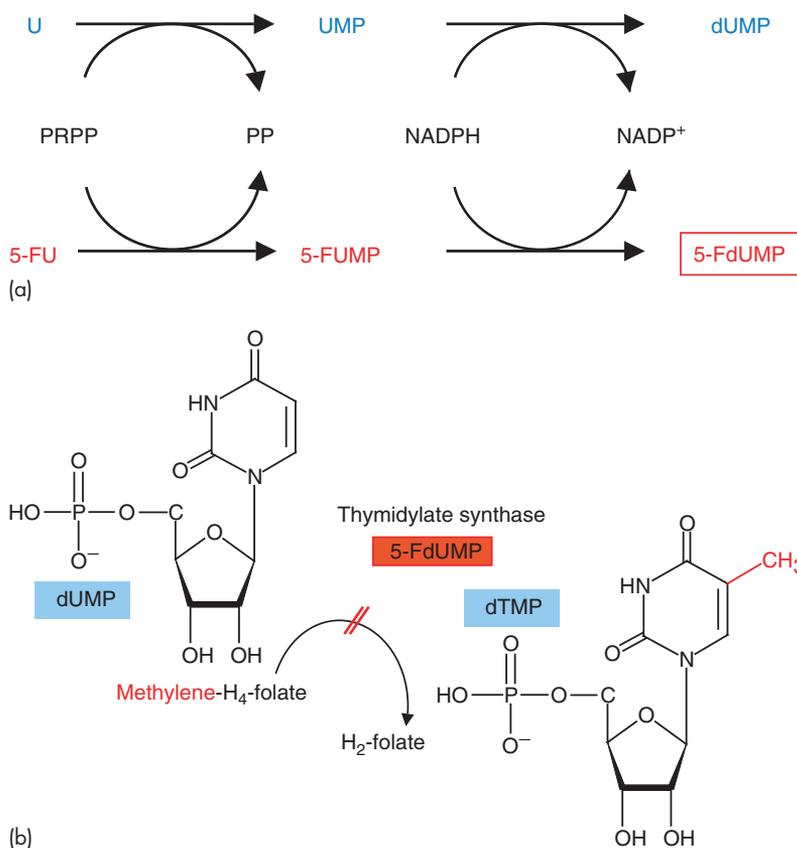**Figure 4.2** Mechanism of action of cytotoxic drugs: methotrexate.

Figure 4.3 Mechanism of action of cytotoxic drugs: Fluorouracil. 5-Fluorouracil (5FU) can participate in many reactions in which uracil would normally be involved. Firstly, it has to be converted to its active form, 5-fluoro-2 deoxyuridine monophophate (5-FdUMP) **(a)**. This then interferes with DNA synthesis by binding to the enzyme thymidylate synthetase, causing it to be inactivated **(b)**. The binding can be stabilized by the addition of folinic acid. 5FdUMP, 5-fluorodeoxyuridine monophosphate; 5FU, 5-fluorouracil; 5FUMP, 5-fluorouridine monophosphate; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophophase; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; PP, pyrophosphate; PRPP, 5-phospho-alpha-D-ribose 1-diphosphate; U, uracil; UMP, uridine monophosphate.

plant. They are mitotic spindle poisons that act by binding to tubulin, the building block of the microtubules. This inhibits further assembly of the spindle during metaphase, thus inhibiting mitosis. Although microtubules are important in other cell functions (hormone secretion, axonal transport and cell motility), it is likely that the influence of this group of drugs on DNA repair contributes most significantly to their toxicity. Other newer examples include vindesine and vinorelbine.

Taxoids

Paclitaxel (Taxol) is a drug derived from the bark of the pacific yew, *Taxus brevifolia*. It promotes assembly of microtubules and inhibits their disassembly. Direct activation of apoptotic pathways has also been suggested to be critical to the cytotoxicity of this drug.[2] Docetaxel (Taxotere) is a semisynthetic derivative.

TOPOISOMERASE INHIBITORS

Topoisomerases are responsible for altering the 3D structure of DNA by a cleaving/unwinding/rejoining reaction. They are involved in DNA replication, chromatid segregation and transcription. It has previously been considered that the efficacy of topoisomerase inhibitors in the treatment of cancer was based solely on their ability to inhibit DNA replication. It has now been suggested that drug efficacy may also depend on the simultaneous manipulation of other cellular pathways within tumour cells.[3] The drugs are phase-specific and prevent cells from entering mitosis from G2. There are two broad classes:

Topoisomerase I inhibitors

Camptothecin, derived from *Camptotheca acuminata* (a Chinese tree), binds to the enzyme–DNA complex, stabilizing it and preventing DNA replication. Irinotecan and topetecan have been derived from this prototype.

Topoisomerase II inhibitors

Epipodophyllotoxin derivatives (e.g. etoposide, vespid) are semisynthetic derivatives of *Podophyllum peltatum*, the American mandrake. They stabilize the complex between

topoisomerase II and DNA that causes strand breaks and ultimately inhibits DNA replication.

LIMITATIONS OF CYTOTOXIC AGENTS

There are a number of problems with the safety profile and efficacy of chemotherapeutic agents. Cytotoxics predominantly affect rapidly dividing cells so do not specifically target cancer cells in the resting phase. They also only influence a cell's ability to divide and have little effect on other aspects of tumour progression such as tissue invasion, metastases or progressive loss of differentiation. Finally, cytotoxics are associated with a high incidence of adverse effects. The most notable examples include bone marrow suppression, alopecia, mucositis, nausea and vomiting.

CHEMOTHERAPY IN HEAD AND NECK CANCER

Worldwide, squamous cell cancer of the head and neck accounts for an estimated 500,000 new cancer cases per year. One-third of these patients present with early stage disease that is amenable to cure with surgery or radiotherapy alone. The remaining patients usually present with locally advanced disease. Unfortunately, this group exhibit high recurrence rates of approximately 65 percent despite radical surgery and radiotherapy. To date, the addition of chemotherapy has not changed this. It has, however, allowed improved organ preservation when combined with radiotherapy and has led to a reduction in rates of distant metastases. Chemotherapy also has a role in the palliative treatment of advanced disease.

Currently, surgery or radiotherapy are the standard curative options for early stage head and neck cancer. Chemotherapy in combination with surgery, radiotherapy or both is employed for locoregionally advanced disease. Stage IV disease is managed with palliative chemotherapy (see also Chapter 200, Developments in radiotherapy for head and neck cancer).

CHOICE OF CHEMOTHERAPY IN HEAD AND NECK CANCER

The single agents active in head and neck cancer, with response rates between 15 and 40 percent, include methotrexate, cisplatin, carboplatin, fluorouracil, ifosfamide, bleomycin, paclitaxel and docetaxel. Cisplatin is particularly popular for use either as a single agent or in combination with other drugs because for a long time it was viewed as one of the most active drugs in squamous head and neck cancer.[4] Taxoids and gemcitabine are now gaining favour and are being incorporated into many current drug trials. [***]

CHEMOTHERAPY STRATEGIES

Combination chemotherapy

Combinations of cytotoxic agents are widely used for many cancers and may be more effective than single agents. Possible explanations for this include:

- exposure to agents with different mechanisms of action and nonoverlapping toxicities;
- reduction in the development of drug resistance;
- the ability to use combinations of drugs that may be synergistic.

In practice, the predominant dose-limiting toxicity of many cytotoxic drugs is myelosuppression and this limits the doses of individual drugs when used in combination.

Adjuvant chemotherapy

This is the use of chemotherapy in patients known to be at risk of relapse by virtue of features determined at the time of definitive local treatment (e.g. tumour grade, lymph node status, etc.). The intention of adjuvant chemotherapy is therefore the eradication of micrometastatic disease.

Randomized trials assessing the use of adjuvant chemotherapy for the patients with head and neck squamous carcinoma do not suggest a significant benefit.[5] [****]

Neoadjuvant chemotherapy

Neoadjuvant, or induction chemotherapy, is the use of chemotherapy before definitive surgery or radiotherapy in patients with locally advanced disease. The intention of this strategy is to improve local and distant control of the disease in order to achieve greater organ preservation and overall survival.

Numerous phase III trials have considered the benefit of neoadjuvant chemotherapy followed by definitive surgery, by surgery and radiotherapy, or by radiotherapy alone as compared to definitive management without chemotherapy. Unfortunately, these studies have not demonstrated a survival advantage. To date, only subset analyses of trials using neoadjuvant cisplatin and 5-fluorouracil combination chemotherapy compared with locoregional treatment alone have shown a small survival gain.[5] In addition, neoadjuvant chemotherapy has been shown to have little impact on reducing locoregional failure. This is perhaps surprising given the consistently observed high initial tumour response rates of up to 70–85 percent.

The role of neoadjuvant chemotherapy therefore continues to remain controversial and further studies are planned, particularly looking at more effective drug combinations. [****]

Concurrent chemoradiation

This involves the synchronous use of chemotherapy and radiotherapy. Multiple randomized trials comparing concurrent radiotherapy and chemotherapy with radiotherapy alone have shown significant improvement in locoregional control, relapse-free survival and overall survival rates in patients with locally advanced, unresectable disease.[6] These results may reflect the influence of chemotherapy on micrometastatic disease or its ability to enhance tumour radiosensitivity.[7] Some chemotherapy agents are recognized to be more active in certain radioresistant cell types. Other drugs may act synergistically with radiotherapy by hindering the repair of radiation-induced DNA damage (cisplatin), by synchronizing or arresting cells during radiosensitive phases (hydroxyurea, paclitaxel) or by hindering regrowth between fractions of treatment.

Many different drug combinations and radiation schedules have been evaluated. Each combination clearly has unique toxicities, risks and benefits. At present, there is still debate regarding the optimum chemoradiotherapy regimen that should become the standard of care. [****/**]

High-dose chemotherapy

Many chemotherapy drugs have a linear dose–response curve, but their use at high doses is limited by myelosuppression. This may be overcome by using bone marrow or peripheral stem cell infusions. While high-dose chemotherapy appears to have a role in the management of leukaemias, myeloma and certain lymphomas, little benefit has been demonstrated in common solid tumours. [****]

Chemoprevention

This is a novel approach with the aim of reversing or halting carcinogenesis with the use of pharmacologic or natural agents. Retinoids have been tested in head and neck carcinogenesis both in animal models and against oral premalignant lesions and in the prevention of secondary tumours in humans, with initial encouraging results.[8, 9] Studies are also looking at the benefit of using cyclo-oxygenase 2 (COX-2) inhibitors in a similar role.[10] [**]

NOVEL THERAPIES FOR THE FUTURE

Despite the introduction of new cytotoxic drugs, such as antimetabolites (capecitabine) and topoisomerase I inhibitors, the management of advanced head and neck cancer remains challenging. Over the last years interest has focussed more on novel agents with a more targeted mechanisms of action.

Targeted therapy aims to specifically act on a well-defined target or biologic pathway that, when inactivated, causes regression or destruction of the malignant process. The main strategies of research have looked at the use of monoclonal antibodies or targeted small molecules.

Monoclonal antibodies

In the early 1980s, it became apparent that targetted therapy using monoclonal antibodies (MAb) might be useful in the detection and treatment of cancer. Monoclonal antibodies can be derived from a variety of sources:

- murine: mouse antibodies;
- chimeric: part mouse/part human antibodies;
- humanized: engineered to be mostly human;
- human: fully human antibodies.

Murine monoclonal antibodies may themselves induce an immune response that may limit repeated administration. Humanized and, to a lesser extent, chimeric antibodies are less immunogenic and can be given repeatedly.

There are several proposed mechanisms of action of monoclonal antibodies.[11] These include:

- direct effects:
  - induction of apoptosis;
  - inhibition of signalling through the receptors needed for cell proliferation/function;
  - anti-idiotype antibody formation, determinants amplifying an immune response to the tumour cell;
- indirect effects:
  - antibody-dependent cellular cytotoxicity (ADCC, conjugating the 'killer cell' to the tumour cell);
  - complement-mediated cellular cytotoxicity (fixation of complement leading to cytotoxicity).

A desirable target for MAbs would have the following properties:

- wide distribution on tumour cells;
- high level of expression;
- bound to tumour, allowing cell lysis;
- absent from normal tissues;
- trigger activation of complement on MAb binding;
- limited antigenic modulation of target.

Antibodies have also been used as vectors for the delivery of drugs and radiopharmaceuticals to a target of tumour cells.

The earliest and most successful clinical use of antibodies in oncology has been for the treatment of haematological malignancies. Interest in the development of antibodies for solid tumours has become increasingly popular, especially with respect to the epidermal and vascular endothelial growth factor receptors.

Epidermal growth factor receptor biology

Epidermal growth factor receptor (EGFR) biology is a 170-kDa transmembrane protein composed of an extracellular ligand-binding domain, a transmembrane lipophilic region and an intracellular protein tyrosine kinase domain (**Figure 4.4**). When a substrate binds to the receptor, the ligand–receptor complex dimerizes and is internalized by the host cell. This activates an intracellular protein kinase by autophosphorylation, which in
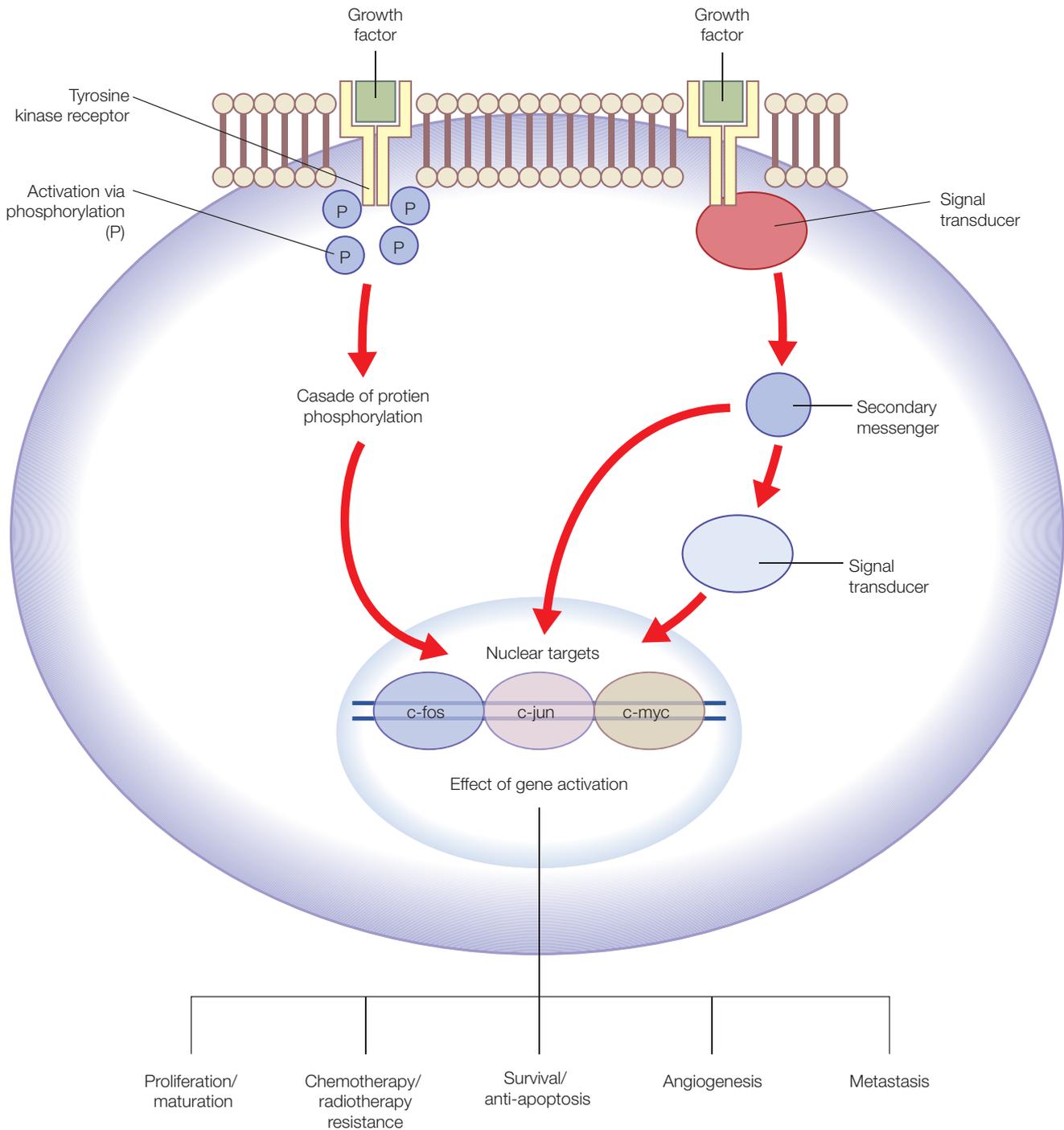

Figure 4.4 Simplified epidermal growth factor receptor signal transduction pathways and opportunities for intervention. GRB2, growth factor receptor binding protein 2; MAPK, mitogen-activated protein kinase; MEK, MAPK/extracellular signal related kinase; SOS, guanine nucleotide exchange factor (son of sevenless); c-fos, c-jun and c-myc, nuclear targets involved in gene transcription/cell cycle progression; P, phosphate; TGFα, transformation growth factor α; PI3-K, phosphotidyinositol 3; AKT, serine/threonine kinase, prosurvival protein; STAT, signal transducing activation of transcription.

turn activates signal transduction pathways, influencing cell function. This can lead to cell proliferation, as well as invasion and metastasis.

Several investigators have described amplification of the EGFR gene and overexpression of the EGFR surface membrane protein in a large number of human cancers, including squamous cell carcinoma of the head and neck.[12]

Overexpression is associated with increased proliferative capacity and metastatic potential and is an independent indicator of poor prognosis.[13] Blockade of the EGFR pathway has been shown to inhibit the proliferation of malignant cells and also appears to influence angiogenesis, cell motility and invasion.[14] Various strategies have been investigated to manipulate EGFR.

Monoclonal antibodies against epidermal growth factor receptor

MAb technology has been directed against EGFR. The chimeric IgG antibody cetuximab (C225) has the binding affinity equal to that of the natural ligand and can effectively block the effect of epidermal growth factor and transforming growth factor α.[6] It has been shown to enhance the antitumour effects of chemotherapy and radiotherapy in preclinical models.[15, 16, 17] More recently, cetuximab has been evaluated alone and in combination with radiotherapy and various cytotoxic chemotherapeutic agents in a series of phase II and III studies involving patients with head and neck cancers.[15, 18] The studies are encouraging, but it is still too early to determine the exact role the antibody will play in treatment regimens.

Targeted small molecules against epidermal growth factor receptor

Gefitinib (Iressa) and erlotinib (Tarceva) are orally active epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) that block the EGFR signalling cascade, thereby inhibiting the growth, proliferation and survival of many solid tumours. They have single agent activity in patients with recurrent or metastatic head and neck cancer, and have an acceptable safety profile compared with conventional chemotherapy.[19, 20] Results of phase III trials are awaited and will help determine their optimal use in head and neck cancers.

Interestingly, one of the noted side effects of the drugs is an acneiform rash. Analysis of phase II trials of erlotinib in nonsmall-cell lung cancer, head and neck cancer and ovarian cancer shows a significant association between the rash severity and objective tumour response and overall survival.[21] Similar findings have been made with cetuximab and gefitinib. This association suggests that the rash may serve as a marker of response to treatment and could be used to guide treatment to obtain optimal dose.

Despite these successes, these agents have modest activity when used as single agents in unselected patients. It is clear that the clinical development of these agents is far from simple. It is important that we try to understand better the biological and clinical criteria for patient selection and also how best to use the different available agents. The recent discovery of EGFR mutations and the potential identification of other markers that might predict patient response could help to optimize the use of these agents in the future.

Inhibitors of angiogenesis

Angiogenesis is the process of new blood vessel formation, triggered by hypoxia and regulated by numerous stimulators and inhibitors (**Figure 4.5**). It is vital for cancer development. A tumour cannot extend beyond 2–3 mm without inducing a vascular supply. New vessels develop on the edge of the tumour and then migrate into the tumour. This process relies on degradation of the extracellular matrix surrounding the tumour by matrix metalloproteinases, such as collagenase, that are expressed at high levels in some tumour and stromal cells. Angiogenesis is then dependent on the migration and proliferation of endothelial cells.

It has been found that antiangiogenic agents tend to be cytostatic rather than cytotoxic, hence stabilizing the tumour and preventing spread. As a consequence, they may be valuable for use in combination with cytotoxic drugs, as maintenance therapy in early-stage cancers or as adjuvant treatment after definitive radiotherapy or surgery. There is evidence to support the fact that suppressing angiogenesis can maintain metastases in a state of dormancy.[22] Interestingly, development of resistance does not appear to be a feature of these drugs.[23] [**]

Vascular endothelial growth factor receptor

Vascular endothelial growth factor is a multifunctional cytokine released in response to hypoxia and is an important stimulator of angiogenesis. It binds to two structurally related trans-membrane receptors present on endothelial cells, called Flt-1 and KDR. High VEGF protein and receptor expression has been demonstrated in certain head and neck cancers and is associated with a higher tumour proliferation rate and worse survival.[24]

Monoclonal antibodies against vascular endothelial growth factor receptor

Bevacizumab (Avastin) is a humanized murine monoclonal antibody targeting VEGF. It is the first antiangiogenic drug to have induced a survival advantage in cancer therapy, within a randomized trial of irinotecan,
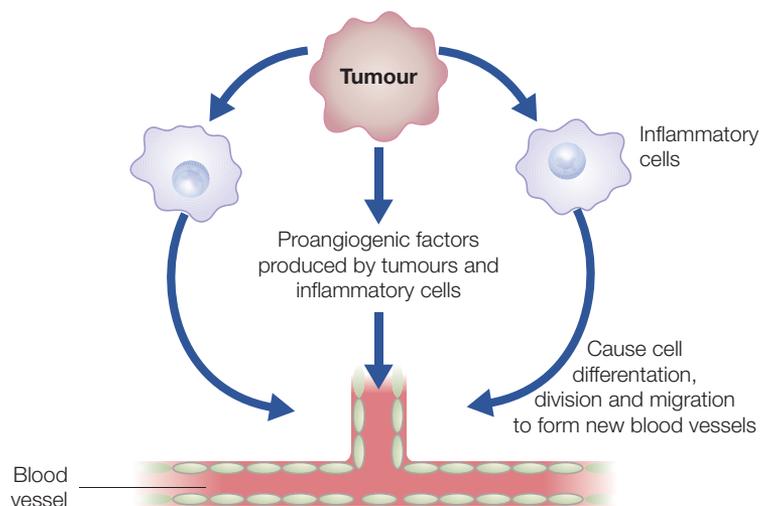
Pro-angiogenic factors	Anti-angiogenic factors
Fibroblast growth factor	Thrombospondin I
Placental growth factor	Angiostatin
Vascular endothelial growth factor	Interferon alpha
Transforming growth factors	Prolactin
Angiogenin	Metallo-proteinase inhibitors
Interleukin-8	Endostatin
Hepatocyte growth factor	Platelet factor 4
Platelet derived enthothelial cell growth factor	Placental profliferin-related protein

Figure 4.5 Schematic representation of possible anti–angiogenesis targets, including natural stimulators and inhibitors.

5-fluorouracil, leucovorin combined with bevacizumab or placebo in metastatic colorectal cancer.[25] The use of bevacizumab in head and neck cancer is supported by data from preclinical studies.[26] Currently, clinical trials are exploring the feasibility and the therapeutic potential of a combination of bevacizumab and EGFR-targeted drugs.[27]

OTHER NOVEL TREATMENTS

There are now a large number of new types of agents entering all phases of clinical trials. To date, they have met with variable success. It is important to mention a few drugs which have really made an impact on treatment of specific cancers in the last few years.

- **Trastuzumab (Herceptin):** A humanized monoclonal antibody against the HER-2 receptor which is now becoming increasingly important in the treatment of both locally advanced and metastatic breast cancer.
- **Imatinib mesylate (Gleevec):** An adenosine triphosphate binding selective inhibitor of bcr-abl that has been shown to produce durable complete haematologic and cytogenetic remissions in early chronic phase CML. It also has remarkable activity against relapsed and metastatic gastrointestinal stromal tumours (GIST) that characteristically feature a mutation in the c-kit receptor tyrosine kinase gene.

- **Ritiximab (Mabthera):** The rituximab antibody is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. It is being increasingly used in combination with chemotherapy to manage many different types of indolent and aggressive B-cell lymphomas.
- **Bortezomib (Velcade):** Velcade is the first of a new class of agents called proteasome inhibitors and the first treatment in more than a decade to be approved for patients with multiple myeloma.

The proteasome is an enzyme complex that exists in all cells and plays an important role in degrading proteins fundamental to all cellular processes, in particular those involved in cell growth and survival. Velcade is a potent but reversible inhibitor of the proteasome. By disrupting normal cellular processes, proteasome inhibition promotes apoptosis. Cancer cells appear to be more susceptible to this effect than normal cells. Due to the reversibility of proteasome inhibition with Velcade, normal cells are more readily able to recover, whereas cancer cells are more likely to undergo apoptosis.

CONCLUSION

The majority of conventional chemotherapeutic agents cause cell death by directly inhibiting the synthesis of

DNA or interfering with its function. This means that they are often not tumour-specific and are associated with considerable morbidity. Trials have demonstrated that combination chemotherapy regimens can cause dramatic regression of head and neck tumours, especially when used concomitantly with radiotherapy. Unfortunately, this has not been associated with an increase in survival rates.

There is considerable excitement over the development of new target-directed cytotoxic agents. These have been developed to modulate or inhibit specific molecular targets critical to the development of or control of cancer cells. Particular interest has focussed on the field of monoclonal antibody development, particularly in relation to the epidermal growth factor. Other drugs affecting signal transduction, programmed cell death, transcription regulation, matrix invasion and angiogenesis are currently involved in clinical trials. The results of these are obviously eagerly awaited and will potentially radically change current therapeutic strategies.

KEY POINTS

- Traditional chemotherapy agents interfere with DNA synthesis and function and are classified according to their mechanism of action.
- Many agents are associated with significant side-effect profiles.
- The role of chemotherapy in head and neck cancer is still being defined, but there is increasing popularity of concurrent chemotherapy and radiotherapy regimens.
- Current research is focussing on molecular targeted therapy.
- Recent strategies have looked at the use of monoclonal antibodies.
- Drugs are being designed that influence signal transduction, specifically cell cycle regulation, apoptosis, matrix invasion and angiogenesis.
- Results of clinical trials are eagerly awaited.

Deficiencies in current knowledge and areas for future research

The effect of chemotherapy on nonmetastatic head and neck cancers is still being elucidated. The optimum combination of chemotherapeutic agents and the timing of their use in relation to surgery have not been defined, especially in combination with radiotherapy. As this continues to be assessed, significant advances are being made in relation to more specific targeted therapies. The results of clinical trials with these new agents and their incorporation into management regimens are eagerly awaited.

➤ The optimum regimen of chemotherapy agents for use in head and neck cancers needs to be defined aiming to improve survival, quality of life and organ function.
➤ The role of molecular target-specific chemotherapy agents in the management of head and neck cancers needs to become more familiar.

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