56 Anticancer drugs

OVERVIEW

In this chapter we deal with cancer[1] and anticancer drug therapy. We discuss first the pathogenesis of cancer and then describe the drugs that can be used to treat malignant disease. Finally, we consider the extent to which our new knowledge of cancer biology is leading to new therapies. The use of radioactive isotopes in cancer treatment is beyond the scope of this book.

INTRODUCTION

'Cancer' is characterised by uncontrolled multiplication and spread of abnormal forms of the body's own cells. It is the second most common cause of death in the developed nations (cardiovascular disease has the dubious distinction of heading that table) and one in three people will be diagnosed with cancer during their lifetime. According to Cancer Research UK (2013), over 325 000 new cases were reported in the UK in 2010 and mortality was in excess of 157 000 (global figure, 7.4 million). Cancer is responsible for approximately one-quarter of all deaths in the UK. Lung and bowel cancer are the commonest malignancies, closely followed by breast and prostate cancer. Statistics from most other countries in the developed world tell much the same story.

A comparison of the incidence of cancer over the past 100 years or so gives the impression that the disease is increasing in developed countries, but this is not so. Cancer occurs mainly in later life and, with advances in public health and medical science, many more people now live to an age where malignancy is common.

The terms *cancer*, *malignancy* and *malignant tumour* are often used synonymously.[2] Both benign and malignant tumours manifest uncontrolled proliferation, but the latter are distinguished by their capacity for *de-differentiation*, their *invasiveness* and their ability to *metastasise* (spread to other parts of the body). In this chapter, we shall be concerned only with the therapy of malignant disease. The appearance of these abnormal characteristics reflects altered patterns of gene expression in the cancer cells, resulting from inherited or acquired mutations.

There are three main approaches to treating established cancer – *surgical excision*, *irradiation* and *drug therapy* (previously often called *chemotherapy*, but now often including hormonal and biological agents as described below and

in Chs 35 and 59) – and the relative value of each of these approaches depends on the disease and the stage of its development. Drug therapy may be used on its own or as an adjunct to other forms of therapy.

Compared with that of bacterial diseases, cancer chemotherapy presents a difficult conceptual problem. In biochemical terms, microorganisms are both quantitatively and qualitatively different from human cells (see Ch. 50), but cancer cells and normal cells are so similar in most respects that it is more difficult to find general, exploitable, biochemical differences between them. Conventional *cytotoxic drugs* act on all cells and rely on a small margin of selectivity to be useful as anticancer agents, but the scope of cancer therapy has now broadened to include drugs that affect either the hormonal regulation of tumour growth, or the defective cell cycle controls that underlie malignancy (see Ch. 5 and Weinberg et al., 1996). Overall, this has been one of the most fruitful fields of drug development in recent years, and both genomics and biopharmaceuticals have played a major role. The flow of innovation seems set to continue.

THE PATHOGENESIS OF CANCER

To understand the action and drawbacks of current anticancer agents and to appreciate the therapeutic hurdles that must be surmounted by putative new drugs, it is important to consider the pathobiology in more detail.

Cancer cells manifest, to varying degrees, four characteristics that distinguish them from normal cells. These are:

- *uncontrolled proliferation*
- *de-differentiation and loss of function*
- *invasiveness*
- *metastasis.*

THE GENESIS OF A CANCER CELL

A normal cell turns into a cancer cell because of one or more mutations in its DNA. These can be inherited or acquired, usually through exposure to viruses or *carcinogens* (e.g. tobacco products, asbestos). A good example is breast cancer; women who inherit a single defective copy of either of the tumour suppressor genes *BRCA1* and *BRCA2* have a significantly increased *risk* of developing breast cancer. However, carcinogenesis is a complex multistage process, usually involving more than one genetic change as well as other, *epigenetic* factors (hormonal, co-carcinogen and tumour promoter effects, etc.) that do not themselves produce cancer but which increase the *likelihood* that the genetic mutation(s) will eventually result in cancer.

There are two main categories of relevant genetic change:

1. The activation of *proto-oncogenes* to *oncogenes*. Proto-oncogenes are genes that normally control cell

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[1]The term 'cancer' actually embraces a range of different diseases, each with its own characteristic aetiology and clinical outcome, but all giving rise to uncontrolled cell growth. Whilst acknowledging this, we have retained this historical category here for convenience.

[2]Blood cell malignancies – lymphomas and leukaemias – are non-tumour-forming, and drot usually referred to as cancers. In this account, 'cancer' is used to cover all malignancies.

division, apoptosis and differentiation (see Ch. 5), but which can be converted to oncogenes that induce malignant change by viral or carcinogen action.

2. The inactivation of *tumour suppressor genes*. Normal cells contain genes that suppress malignant change – termed tumour suppressor genes (*anti-oncogenes*) – and mutations of these genes are involved in many different cancers. The loss of function of tumour suppressor genes can be the critical event in carcinogenesis.

About 30 tumour suppressor genes and 100 dominant oncogenes have been identified. The changes that lead to malignancy are a result of point mutations, gene amplification or chromosomal translocation, often caused by viruses or chemical carcinogens.

THE SPECIAL CHARACTERISTICS OF CANCER CELLS

UNCONTROLLED PROLIFERATION

It is not generally true that cancer cells proliferate faster than normal cells. Many healthy cells, in the bone marrow and the epithelium of the gastrointestinal tract (for example), undergo continuous rapid division. Some cancer cells multiply slowly (e.g. those in plasma cell tumours) and some much more rapidly (e.g. the cells of *Burkitt's lymphoma*). The significant issue is that cancer cells *have escaped from the mechanisms that normally regulate cell division and tissue growth*. It is this, rather than their rate of proliferation, that distinguishes them from normal cells.

What are the changes that lead to the uncontrolled proliferation of tumour cells? Inactivation of tumour suppressor genes or transformation of proto-oncogenes into oncogenes can confer autonomy of growth on a cell and thus result in uncontrolled proliferation by producing changes in cellular systems (see Fig. 56.1), including:

- *growth factors*, their receptors and signalling pathways
- the *cell cycle transducers*, for example cyclins, cyclin-dependent kinases (cdks) or the cdk inhibitors
- the *apoptotic machinery* that normally disposes of abnormal cells
- *telomerase expression*
- *local blood vessels*, resulting from tumour-directed angiogenesis.

Potentially all the genes coding for the above components could be regarded as oncogenes or tumour suppressor genes (see Fig. 56.2), although not all are equally prone to malignant transformation. It should be understood that
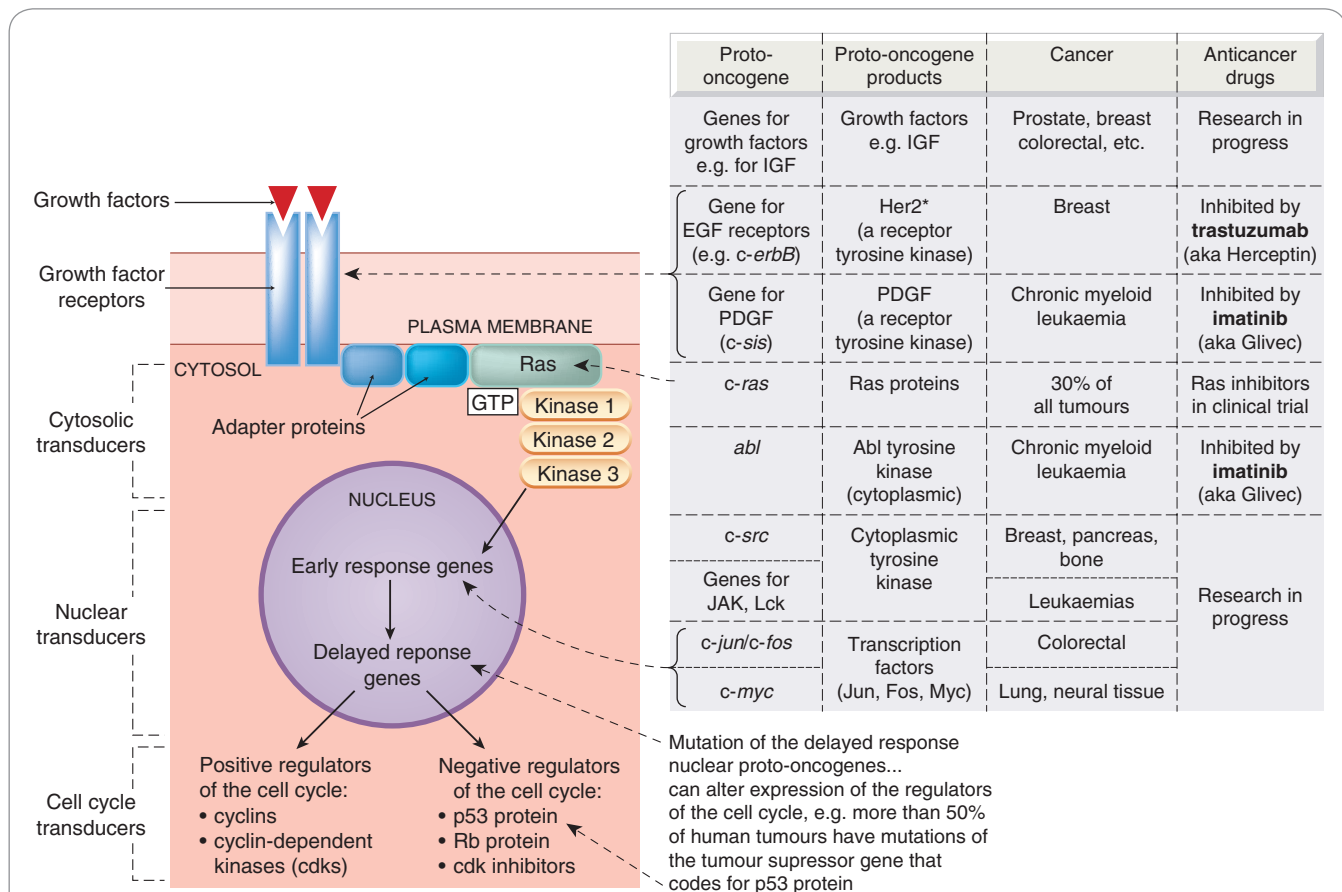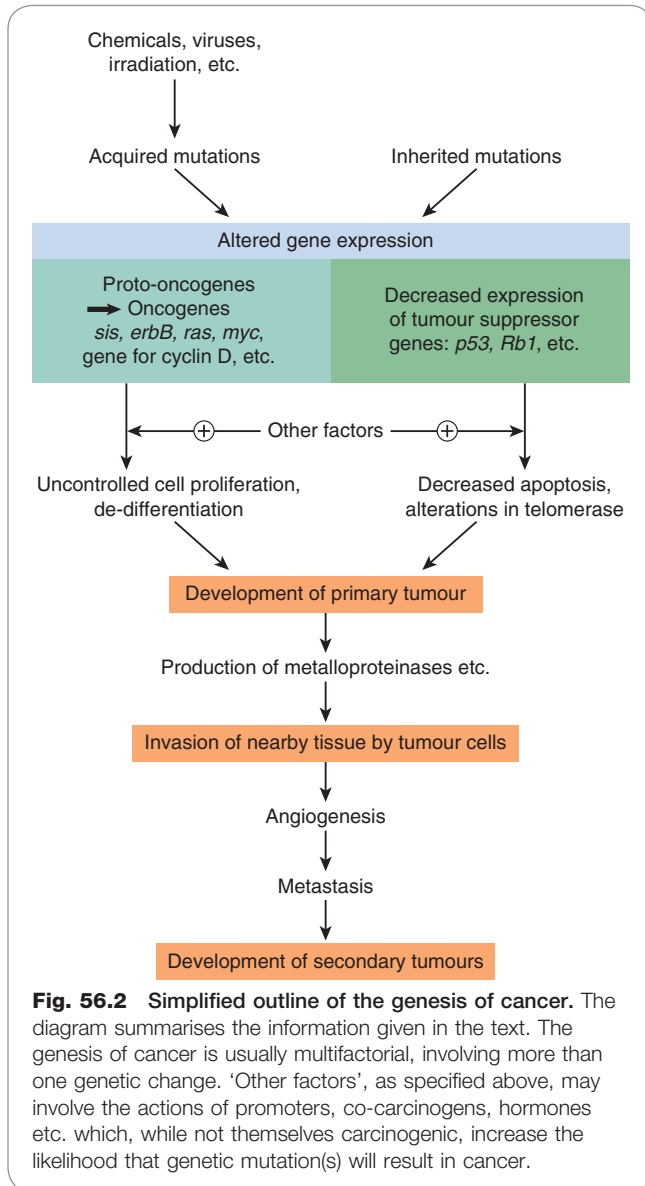

Proto-oncogene	Proto-oncogene products	Cancer	Anticancer drugs
Genes for growth factors e.g. for IGF	Growth factors e.g. IGF	Prostate, breast colorectal, etc.	Research in progress
Gene for EGF receptors (e.g. c-*erbB*)	Her2* (a receptor tyrosine kinase)	Breast	Inhibited by **trastuzumab** (aka Herceptin)
Gene for PDGF (c-*sis*)	PDGF (a receptor tyrosine kinase)	Chronic myeloid leukaemia	Inhibited by **imatinib** (aka Glivec)
c-*ras*	Ras proteins	30% of all tumours	Ras inhibitors in clinical trial
abl	Abl tyrosine kinase (cytoplasmic)	Chronic myeloid leukaemia	Inhibited by **imatinib** (aka Glivec)
c-*src*	Cytoplasmic tyrosine kinase	Breast, pancreas, bone	Research in progress
Genes for JAK, Lck		Leukaemias	
c-*jun*/c-*fos*	Transcription factors (Jun, Fos, Myc)	Colorectal	
c-*myc*		Lung, neural tissue	

Fig. 56.1 **Signal transduction pathways initiated by growth factors and their relationship to cancer development.** A few examples of proto-oncogenes and the products they code for are given in the table, with examples of the cancers that are associated with their conversion to oncogenes. Many growth factor receptors are receptor tyrosine kinases, the cytosolic transducers including adapter proteins that bind to phosphorylated tyrosine residues in the receptors. Ras proteins are guanine nucleotide-binding proteins and have GTPase action; decreased GTPase action means that Ras remains activated. EGF, epidermal growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; *Her2* is also termed *her2/neu*.

Chemicals, viruses,
irradiation, etc.

↓

Acquired mutations   Inherited mutations

↓   ↙

Altered gene expression

| Proto-oncogenes → Oncogenes *sis, erbB, ras, myc*, gene for cyclin D, etc. | Decreased expression of tumour suppressor genes: *p53, Rb1*, etc. |

↓ ←—— ⊕ — Other factors — ⊕ —→ ↓

Uncontrolled cell proliferation,   Decreased apoptosis,
de-differentiation   alterations in telomerase

↓   ↓

Development of primary tumour

↓

Production of metalloproteinases etc.

↓

Invasion of nearby tissue by tumour cells

↓

Angiogenesis

↓

Metastasis

↓

Development of secondary tumours

Fig. 56.2 **Simplified outline of the genesis of cancer.** The diagram summarises the information given in the text. The genesis of cancer is usually multifactorial, involving more than one genetic change. 'Other factors', as specified above, may involve the actions of promoters, co-carcinogens, hormones etc. which, while not themselves carcinogenic, increase the likelihood that genetic mutation(s) will result in cancer.

malignant transformation of several components is needed for the development of cancer.

Resistance to apoptosis
Apoptosis is programmed cell death (Ch. 5), and mutations in antiapoptotic genes are usually a prerequisite for cancer; indeed, resistance to apoptosis is a hallmark of malignant disease. It can be brought about by inactivation of proapoptotic factors or by activation of antiapoptotic factors.

Telomerase expression
Telomeres are specialised structures that cap the ends of chromosomes – like the small metal tubes on the end of shoelaces – protecting them from degradation, rearrangement and fusion with other chromosomes. Furthermore, DNA polymerase cannot easily duplicate the last few nucleotides at the ends of DNA, and telomeres prevent loss of the 'end' genes. With each round of cell division, a portion of the telomere is eroded, so that eventually it becomes non-functional. At this point, DNA replication ceases and the cell becomes senescent.

Rapidly dividing cells, such as stem and bone marrow cells, the germline and the epithelium of the gastrointestinal tract, express *telomerase*, an enzyme that maintains and stabilises telomeres. While it is absent from most fully differentiated somatic cells, about 95% of late-stage malignant tumours do express this enzyme, and it is this that may confer 'immortality' on cancer cells (see Buys, 2000; Keith et al., 2004).

The control of tumour-related blood vessels
The factors described above lead to the uncontrolled proliferation of individual cancer cells, but other factors, particularly blood supply, determine the actual growth of a solid tumour. Tumours 1–2 mm in diameter can obtain nutrients by diffusion, but their further expansion requires *angiogenesis*, the development of new blood vessels in response to growth factors produced by the growing tumour (see Griffioen & Molema, 2000).

DE-DIFFERENTIATION AND LOSS OF FUNCTION

The multiplication of normal cells in a tissue begins with division of the undifferentiated stem cells giving rise to *daughter cells* that differentiate to become the mature non-dividing cells, ready to perform functions appropriate to that tissue. For example, mature fibroblasts secrete and organise extracellular matrix; mature muscle cells are capable of contraction etc. One of the main characteristics of cancer cells is that they de-differentiate to varying degrees. In general, poorly differentiated cancers multiply faster and carry a worse prognosis than well-differentiated cancers.

INVASIVENESS

Normal cells, other than those of the blood and lymphoid tissues, are not generally found outside their 'designated' tissue of origin. This is because, during differentiation and tissue or organ growth, they develop certain spatial relationships with respect to each other. These relationships are maintained by various tissue-specific survival factors that prevent apoptosis (see Ch. 5). In this way, any cells that escape accidentally lose these survival signals and die.

For example, whilst the cells of the normal mucosal epithelium of the rectum proliferate continuously as the lining is shed, they remain as a lining epithelium. A cancer of the rectal mucosa, in contrast, invades other surrounding tissues. Cancer cells have not only lost, through mutation, the restraints that act on normal cells, but they also secrete enzymes (e.g. metalloproteinases; see Ch. 5) that break down the extracellular matrix, enabling them to move around.

METASTASIS

Metastases are *secondary tumours* ('secondaries') formed by cells that have been released from the initial or *primary tumour* and which have reached other sites through blood vessels or lymphatics, by transportation on other cells or as a result of being shed into body cavities. Metastases are the principal cause of mortality and morbidity in most solid tumours and constitute a major problem for cancer therapy (see Chambers et al., 2002).

As discussed above, displacement or aberrant migration of normal cells would lead to programmed cell death as a result of withdrawal of the necessary antiapoptotic factors. Cancer cells that metastasise have undergone a series of genetic changes that alter their responses to the

regulatory factors that control the cellular architecture of normal tissues, enabling them to establish themselves 'extraterritorially'. Tumour-induced growth of new blood vessels locally favours metastasis.

Secondary tumours occur more frequently in some tissues than in others. For example, metastases of mammary cancers are often found in lung, bone and brain. The reason for this is that breast cancer cells express chemokine receptors such as CXCR4 (see Ch. 18) on their surfaces, and chemokines that recognise these receptors are expressed at high level in these tissues but not in others (e.g. kidney), facilitating the selective accumulation of cells at these sites.

GENERAL PRINCIPLES OF CYTOTOXIC ANTICANCER DRUGS

In experiments with rapidly growing transplantable leukaemias in mice, it has been found that a given therapeutic dose of a cytotoxic drug[3] destroys a constant fraction of the malignant cells. If used to treat a tumour with 10^{11} cells, a dose of drug that kills 99.99% of cells will still leave 10 million (10^7) viable malignant cells. As the same principle holds for fast-growing tumours in humans, schedules for chemotherapy are aimed at producing as near a total cell kill as possible because, in contrast to the situation that occurs in microorganisms, little reliance can be placed on the host's immunological defence mechanisms against the remaining cancer cells. If a tumour is removed (or at least *de-bulked*) surgically, any remaining *micro-metastases* are highly sensitive to chemotherapy, hence its use as adjuvant therapy in these circumstances.

One of the major difficulties in treating cancer is that tumour growth is usually far advanced before cancer is diagnosed. Let us suppose that a tumour arises from a single cell and that the growth is exponential, as it may well be during the initial stages. 'Doubling' times vary, being, for example, approximately 24 h with Burkitt's lymphoma, 2 weeks in the case of some leukaemias, and 3 months with mammary cancers. Approximately 30 doublings would be required to produce a cell mass with a diameter of 2 cm, containing 10^9 cells. Such a tumour is within the limits of diagnostic procedures, although it could easily go unnoticed. A further 10 doublings would produce 10^{12} cells, a tumour mass that is likely to be lethal, and which would measure about 20 cm in diameter if it were one solid mass.

However, continuous exponential growth of this sort does not usually occur. In the case of most solid tumours, as opposed to *leukaemias* (tumours of white blood cells), the growth rate falls as the neoplasm grows. This is partly because the tumour outgrows its blood supply, and partly because not all the cells proliferate continuously. The cells of a solid tumour can be considered as belonging to three compartments:

1. *Compartment A* consists of dividing cells, possibly being continuously in the cell cycle.

2. *Compartment B* consists of resting cells (G_0 phase) which, although not dividing, are potentially able to do so.
3. *Compartment C* consists of cells that are no longer able to divide but which contribute to the tumour volume.

Essentially, only cells in *compartment A*, which may form as little as 5% of some solid tumours, are susceptible to the main current cytotoxic drugs. The cells in *compartment C* do not constitute a problem, but the existence of *compartment B* makes cancer chemotherapy difficult, because these cells are not very sensitive to cytotoxic drugs and are liable to re-enter *compartment A* following chemotherapy.

Most current anticancer drugs, particularly cytotoxic agents, affect only one characteristic aspect of cancer cell biology – cell division – but have no specific inhibitory effect on invasiveness, the loss of differentiation or the tendency to metastasise. In many cases, the antiproliferative action results from an action during S phase of the cell cycle, and the resultant damage to DNA initiates apoptosis. Furthermore, because their main target is cell division, they will affect all rapidly dividing normal tissues, and therefore are likely to produce, to a greater or lesser extent, the following general toxic effects:

- *bone marrow toxicity* (myelosuppression) with decreased leukocyte production and thus decreased resistance to infection
- *impaired wound healing*
- *loss of hair* (alopecia)
- damage to *gastrointestinal epithelium* (including oral mucous membranes)
- *depression of growth* in children
- *sterility*
- *teratogenicity*
- *carcinogenicity* – because many cytotoxic drugs are mutagens.

Rapid cell destruction also entails extensive purine catabolism, and urates may precipitate in the renal tubules and cause kidney damage. Finally, in addition to specific toxic effects associated with individual drugs, virtually all cytotoxic drugs produce severe nausea and vomiting, an 'inbuilt deterrent' now thankfully largely overcome by modern antiemetic drug prophylaxis (Ch. 30).

ANTICANCER DRUGS

The main anticancer drugs can be divided into the following general categories:

- *Cytotoxic drugs*. These include:
  - *alkylating agents* and related compounds, which act by forming covalent bonds with DNA and thus impeding replication
  - *antimetabolites*, which block or subvert one or more of the metabolic pathways involved in DNA synthesis
  - *cytotoxic antibiotics*, i.e. substances of microbial origin that prevent mammalian cell division
  - *plant derivatives* (e.g. vinca alkaloids, taxanes, camptothecins): most of these specifically affect microtubule function and hence the formation of the mitotic spindle.

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[3]The term *cytotoxic drug* applies to any drug that can damage or kill cells. In practice, it is used more restrictively to refer to drugs that inhibit cell division and are therefore potentially useful in cancer chemotherapy.

Cancer pathogenesis and cancer chemotherapy: general principles

- Cancer arises as a result of a series of genetic and epigenetic changes, the main genetic lesions being:
  - inactivation of tumour suppressor genes
  - the activation of oncogenes (mutation of the normal genes controlling cell division and other processes).
- Cancer cells have four characteristics that distinguish them from normal cells:
  - uncontrolled proliferation
  - loss of function because of lack of capacity to differentiate
  - invasiveness
  - the ability to metastasise.
- Cancer cells have uncontrolled proliferation often because of changes in:
  - growth factors and/or their receptors
  - intracellular signalling pathways, particularly those controlling the cell cycle and apoptosis
  - telomerase expression.
- Proliferation may be supported by tumour-related angiogenesis.
- Most anticancer drugs are antiproliferative – most damage DNA and thereby initiate apoptosis. They also affect rapidly dividing normal cells and are thus likely to depress bone marrow, impair healing and depress growth. Most cause nausea, vomiting, sterility, hair loss and teratogenicity.

Fig. 56.3 The effects of bifunctional alkylating agents on DNA. Note the cross-linking of two guanines. A, adenine; C, cytosine; G, guanine; T, thymine.

- *Hormones*, of which the most important are steroids (e.g. glucocorticoids, Ch. 33) as well as drugs that suppress oestrogen synthesis (e.g. aromatase inhibitors) or the secretion of male sex hormones (e.g. gonadorelin analogues, Ch. 35) or antagonise hormone action (e.g. oestrogen and androgen antagonists, Ch. 35).
- *Protein kinase inhibitors:* these drugs inhibit the protein kinases (usually tyrosine kinases but sometimes others) involved in growth factor receptor signal transduction. They are increasingly used in a range of specific malignancies (see Krause & van Etten, 2005).
- *Monoclonal antibodies*: of growing importance in particular types of cancer.
- *Miscellaneous agents* that do not easily fit into the above categories.

The clinical use of anticancer drugs is the province of the specialist, who selects treatment regimens appropriate to the patient with the objective of curing, prolonging life or providing palliative therapy.[4] There are over 80 drugs available in the UK for this purpose and they are often used in combination. The principal treatments are listed in Table 56.1. For reasons of space, we restrict our discussion of mechanisms of action to common examples from each group. A textbook (Airley, 2009) provides detailed information.

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[4]You will have gathered that many anticancer drugs are toxic. 'To be an oncologist,' one practitioner commented, 'one has to hate cancer more than one loves life.'
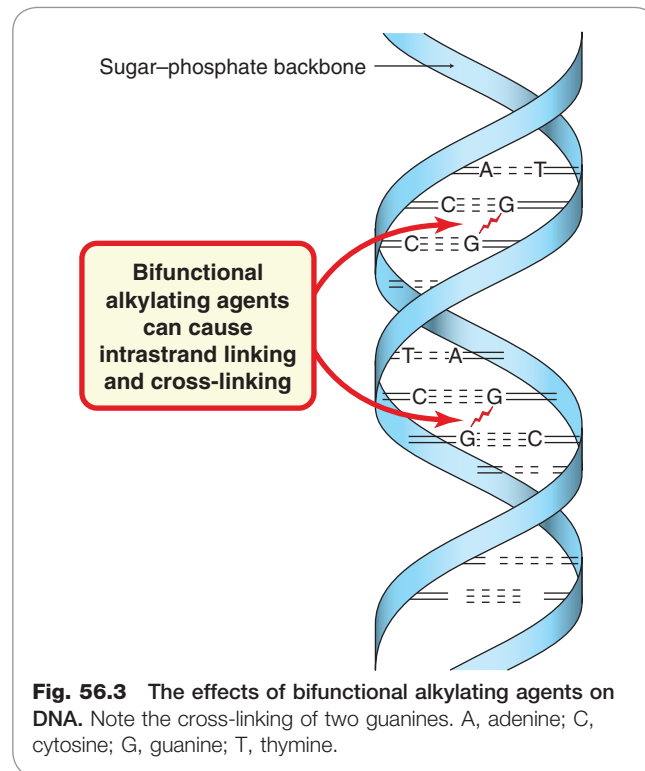
ALKYLATING AGENTS AND RELATED COMPOUNDS

Alkylating agents and related compounds contain chemical groups that can form covalent bonds with particular nucleophilic substances in the cell (such as DNA). With alkylating agents themselves, the main step is the formation of a *carbonium ion* – a carbon atom with only six electrons in its outer shell. Such ions are highly reactive and react instantaneously with an electron donor such as an amine, hydroxyl or sulfhydryl group. Most of the cytotoxic anticancer alkylating agents are *bifunctional*, i.e. they have two alkylating groups (Fig. 56.3).

▼ The nitrogen at position 7 (N7) of guanine, being strongly nucleophilic, is probably the main molecular target for alkylation in DNA (Fig. 56.3), although N1 and N3 of adenine and N3 of cytosine may also be affected. A bifunctional agent, by reacting with two groups, can cause intra- or inter-chain cross-linking. This interferes not only with transcription, but also with DNA replication, which is probably the critical effect of anticancer alkylating agents. Other effects of alkylation at guanine N7 are excision of the guanine base with main chain scission, or pairing of the alkylated guanine with thymine instead of cytosine, and eventual substitution of the GC pair by an AT pair. Their main impact is seen during replication (S phase), when some zones of the DNA are unpaired and more susceptible to alkylation. This results in a block at G_2 and subsequent apoptotic cell death.

All alkylating agents depress bone marrow function and cause hair loss and gastrointestinal disturbances. With prolonged use, two further unwanted effects occur: depression of gametogenesis (particularly in men), leading to sterility, and an increased risk of acute non-lymphocytic leukaemia and other malignancies.

Alkylating agents are among the most commonly employed of all anticancer drugs (about 20 are approved in the UK at the time of writing). Only a few commonly used examples will be dealt with here.

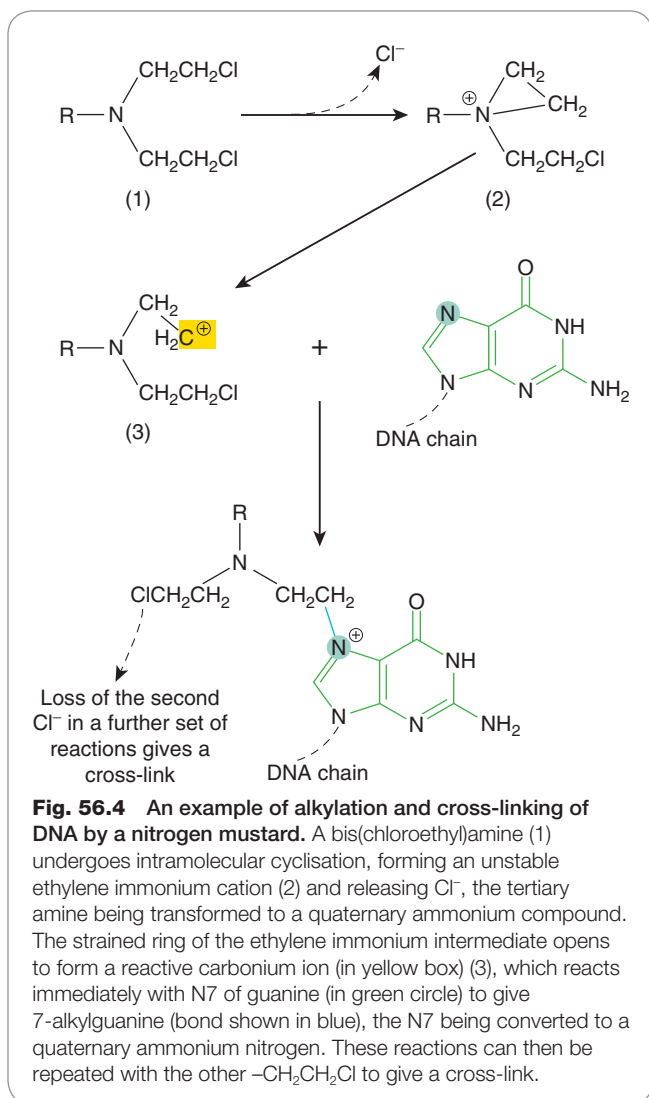**Table 56.1 An overview of anticancer drugs**

Type	Group	Examples	Main mechanism
Alkylating, and related, agents	Nitrogen mustards	Bendramustine, chlorambucil, cyclophosphamide, estramustine,[a] ifosfamide, melphalan	Intrastrand cross-linking of DNA
	Nitrosoureas	Carmustine, lomustine	
	Platinum compounds	Carboplatin, cisplatin, oxaliplatin	
	Other	Busulfan, dacarbazine, hydroxycarbamide, mitobronitol, procarbazine treosulfan, thiotepa, temozolimide	
Antimetabolites	Folate antagonists	Methotrexate, pemetrexed, raltitrexed	Blocking the synthesis of DNA and/or RNA
	Pyrimdine pathway	Azacitidine, capecitabine, cytarabine, decitabine, fluorouracil gemcitabine, tegafur	
	Purine pathway	Cladibrine, clofarabrine, fludarabine, mercaptopurine, nelarabine, pentostatin, tioguanine	
Cytotoxic antibiotics	Anthracyclines	(Amascrine), daunorubicin, doxorubicin, epirubicin, idarubicin, (mitoxantrine)	Multiple effects on DNA/RNA synthesis and topisomerase action
	Other	Bleomycin, dactinomycin, mitomycin, trabectedin	
Plant derivatives and similar compounds	Taxanes	Cabazitaxel, docetaxel, paclitaxel	Microtubule assembly; prevents spindle formation
	Vinca alkaloids	Vinblastine, vincristine, vindesine, vinflunine, vinorelbine. (eribulin)	
	Campothecins	Irinotecan, topotecan	Inhibition of topoisomerase
	Other	Etoposide	
Hormones/ antagonists	Hormones/analogues	Buserelin, diethylstilbestrol, ethinyloestradiol, goserelin, histrelin, lanreotide, leuporelin, medroxyprogesterone, megesterol, norhisterone, triptorelin, octreotide, pasreotide	Act as physiological agonists, antagonists or hormone synthesis inhibitors to disrupt hormone-dependent tumour growth
	Antagonists	Bicalutamide, cyproterone, degarelix, flutamide, fulvestrant, mitotane, tamoxifen, toremifine	
	Aromatase inhibitors	Anastrozole, exemastine, letrozole	
Protein kinase inhibitors	Tyrosine, or other kinase, inhibitors	Axitinib, crizotinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, pazopanib, ruxolitinib, sunitinib, vandetanib, vemurafenib	Inhibition of kinases involved in growth factor receptor transduction
	Pan kinase inhibitors	Everolimus, sorafenib, temsirolimus	
Monoclonal antibodies	Anti-EGF, EGF-2	Panitumumab, trastuzumab	Blocks cell proliferation
	Anti-CD20/CD30/ CD52	Brentixumab, ofatumab, rituximab	Inhibition of lymphocyte proliferation
	Anti-CD3/EpCAM or CTLA-4	Catumexomab	Binds adhesion molecules promoting cell killing
	Anti-VEGF	Bevacizumab	Prevents angiogenesis
Miscellaneous	Retinoid X receptor antagonist	Bexarotene	Inhibits cell proliferation and differentiation
	Proteasome inhibitor	Bortezemib	Activation of programmed cell death
	Enzyme	Cristantaspase	Depletes asparagine
	Photoactivated cytotoxics	Porfimer, temoporfin	Accumulate in cells and kills them when activated by light

[a]A combination of oestrogen and chlormethine. Drugs in parentheses have similar pharmacological actions but are not necessarily chemically related.

Nitrogen mustards

Nitrogen mustards are related to the 'mustard gas' used during the First World War,[5] their basic formula (R-*N-bis*-(2-chloroethyl)) is shown in Figure 56.4. In the body, each 2-chloroethyl side-chain undergoes an intramolecular cyclisation with the release of a Cl^-. The highly reactive *ethylene immonium* derivative so formed can interact with DNA (see Figs 56.3 and 56.4) and other molecules.

Cyclophosphamide is probably the most commonly used alkylating agent. It is inactive until metabolised in the liver by the P450 mixed function oxidases (see Ch. 9). It has a pronounced effect on lymphocytes and can also be used as an immunosuppressant (see Ch. 26). It is usually given orally or by intravenous injection. Important toxic effects are nausea and vomiting, bone marrow depression and haemorrhagic cystitis. This last effect (which also occurs with the related drug **ifosfamide**) is caused by the metabolite acrolein and can be ameliorated

Fig. 56.4 An example of alkylation and cross-linking of DNA by a nitrogen mustard. A bis(chloroethyl)amine (1) undergoes intramolecular cyclisation, forming an unstable ethylene immonium cation (2) and releasing Cl^-, the tertiary amine being transformed to a quaternary ammonium compound. The strained ring of the ethylene immonium intermediate opens to form a reactive carbonium ion (in yellow box) (3), which reacts immediately with N7 of guanine (in green circle) to give 7-alkylguanine (bond shown in blue), the N7 being converted to a quaternary ammonium nitrogen. These reactions can then be repeated with the other –CH_2CH_2Cl to give a cross-link.

[5]It was the clinical insight of Alfred Goodman and Louis Gilman that led to the testing of (what became the first effective anticancer drug) mustine, a modified and stable version of 'mustard gas', to treat lymphomas. They also wrote what was to become a famous textbook of pharmacology.

by increasing fluid intake and administering compounds that are sulfhydryl donors, such as **N-acetylcysteine** or **mesna** (sodium-2-mercaptoethane sulfonate). These agents react with acrolein, forming a non-toxic compound. (See also Chs 9 and 57.)

▼ Other nitrogen mustards used include **bendramustine**, ifosfamide, **chlorambucil** and **melphalan**. **Estramustine** is a combination of **chlormethine** (mustine) with an oestrogen. It has both cytotoxic and hormonal action, and is used for the treatment of prostate cancer.

Nitrosoureas

Examples include **lomustine** and **carmustine**. As they are lipid soluble and cross the blood–brain barrier, they are used to treat tumours of the brain and meninges. However, most nitrosoureas have a severe cumulative depressive effect on the bone marrow that starts 3–6 weeks after initiation of treatment.

Other alkylating agents

Busulfan has a selective effect on the bone marrow, depressing the formation of granulocytes and platelets in low dosage and of red cells in higher dosage. It has little or no effect on lymphoid tissue or the gastrointestinal tract. It is used in chronic granulocytic leukaemia.

Dacarbazine, a prodrug, is activated in the liver, and the resulting compound is subsequently cleaved in the target cell to release an alkylating derivative. Unwanted effects include myelotoxicity and severe nausea and vomiting. **Temozolomide** is a related compound with a restricted usage (malignant glioma).

Procarbazine inhibits DNA and RNA synthesis and interferes with mitosis at interphase. Its effects may be mediated by the production of active metabolites. It is given orally, and its main use is in Hodgkin's disease. It causes **disulfiram**-like actions with alcohol (see Ch. 49), exacerbates the effects of central nervous system depressants and, because it is a weak monoamine oxidase inhibitor, can produce hypertension if given with certain sympathomimetic agents (see Ch. 47). Other alkylating agents in clinical use include **hydroxycarbamide**, **mitobronitol**, **thiotepa** and **treosulfan**.

Platinum compounds

Cisplatin is a water-soluble planar coordination complex containing a central platinum atom surrounded by two chlorine atoms and two ammonia groups. Its action is analogous to that of the alkylating agents. When it enters the cell, Cl^- dissociates, leaving a reactive complex that reacts with water and then interacts with DNA. It causes intra-strand cross-linking, probably between N7 and O6 of adjacent guanine molecules, which results in local denaturation of DNA.

Cisplatin has revolutionised the treatment of solid tumours of the testes and ovary. Therapeutically, it is given by slow intravenous injection or infusion. It is seriously nephrotoxic, and strict regimens of hydration and diuresis must be instituted. It has low myelotoxicity but causes very severe nausea and vomiting. The 5-HT$_3$ receptor antagonists (e.g. **ondansetron**; see Chs 15, 30 and 39) are very effective in preventing this and have transformed cisplatin-based chemotherapy. Tinnitus and hearing loss in the high-frequency range may occur, as may peripheral neuropathies, hyperuricaemia and anaphylactic reactions.

▼ **Carboplatin** is a derivative of cisplatin. Because it causes less nephrotoxicity, neurotoxicity, ototoxicity, nausea and vomiting than

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cisplatin (although it is more myelotoxic), it is sometimes given on an outpatient basis. **Oxaliplatin** is another platinum-containing compound with a restricted application.

ANTIMETABOLITES

Folate antagonists

The main folate antagonist is **methotrexate**, one of the most widely used antimetabolites in cancer chemotherapy. Folates are essential for the synthesis of purine nucleotides and thymidylate, which in turn are essential for DNA synthesis and cell division. (This topic is also dealt with in Chs 25, 50 and 54.) The main action of the folate antagonists is to interfere with thymidylate synthesis.

▼ Structurally, folates consist of three elements: a pteridine ring, *p*-aminobenzoic acid and glutamic acid (Fig. 56.5). Folates are actively taken up into cells, where they are converted to polyglutamates. In order to act as coenzymes, folates must be reduced to tetrahydrofolate (FH_4). This two-step reaction is catalysed by dihydrofolate reductase, which converts the substrate first to dihydrofolate (FH_2), then to FH_4 (Fig. 56.6). FH_4 functions as an essential co-factor carrying the methyl groups necessary for the transformation of 2'-deoxyuridylate (DUMP) to the 2'-deoxythymidylate (DTMP) required for the synthesis of DNA and purines. During the formation of DTMP from DUMP, FH_4 is converted back to FH_2, enabling the cycle to repeat. Methotrexate has a higher affinity than FH_2 for dihydrofolate reductase and thus inhibits the enzyme (Fig. 56.6), depleting intracellular FH_4. The binding of methotrexate to dihydrofolate reductase involves an additional bond not present when FH_2 binds. The reaction most sensitive to FH_4 depletion is DTMP formation.

Methotrexate is usually given orally but can also be given intramuscularly, intravenously or intrathecally. It has low lipid solubility and thus does not readily cross the blood–brain barrier. It is, however, actively taken up into cells by the folate transport system and is metabolised to polyglutamate derivatives, which are retained in the cell for weeks or months even in the absence of extracellular drug. Resistance to methotrexate may develop in tumour cells by a variety of mechanisms. Methotrexate is also used as an immunosuppressant drug to treat rheumatoid arthritis, psoriasis and other autoimmune conditions (see Ch. 26).

Unwanted effects include depression of the bone marrow and damage to the epithelium of the gastrointestinal tract. Pneumonitis can occur. In addition, high-dose regimens – doses 10 times greater than the standard doses, sometimes used in patients with methotrexate

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Anticancer drugs: alkylating agents and related compounds

- Alkylating agents have groups that form covalent bonds with cell substituents; a carbonium ion is the reactive intermediate. Most have two alkylating groups and can cross-link DNA. This causes defective replication and chain breakage.
- Their principal effect occurs during DNA synthesis and the resulting damage triggers apoptosis.
- Unwanted effects include myelosuppression, sterility and risk of non-lymphocytic leukaemia.
- The main alkylating agents are:
  – nitrogen mustards, for example **cyclophosphamide**, which is converted to phosphoramide mustard (the cytotoxic molecule); **cyclophosphamide** myelosuppression affects particularly the lymphocytes
  – nitrosoureas, for example **lomustine**, may act on non-dividing cells, can cross the blood–brain barrier and cause delayed, cumulative myelotoxicity.
- Platinum compounds (e.g. **cisplatin**) cause intrastrand linking in DNA. **Cisplatin** has low myelotoxicity but causes severe nausea and vomiting, and can be nephrotoxic. It has revolutionised the treatment of germ cell tumours.
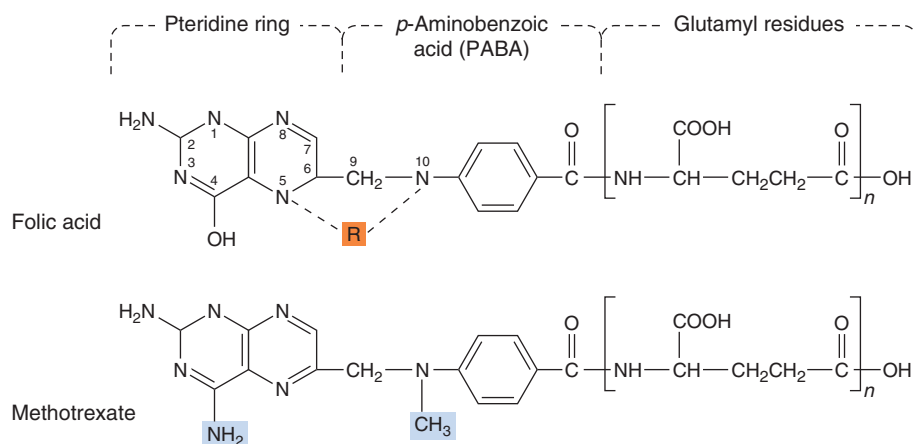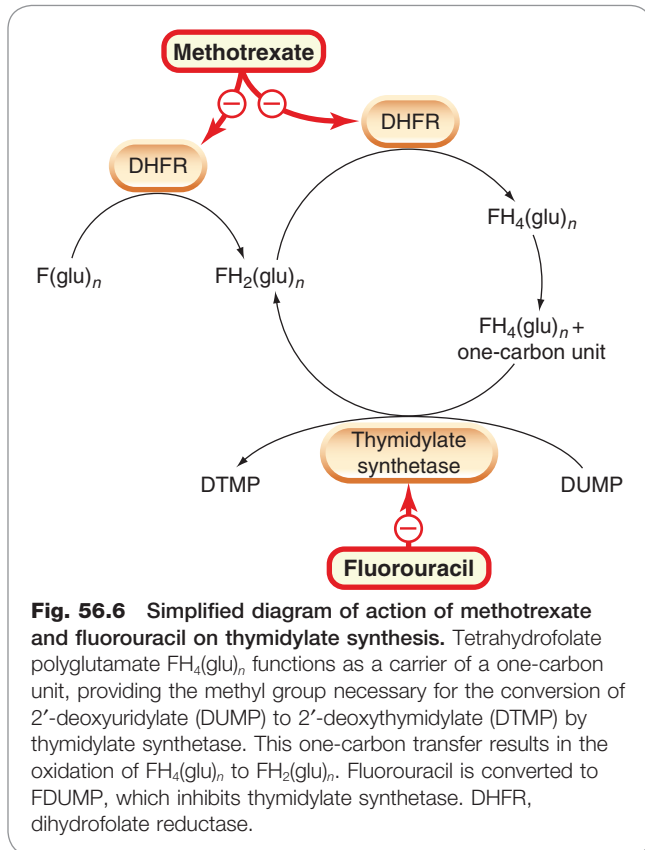

Fig. 56.5 Structure of folic acid and methotrexate. Both compounds are shown as polyglutamates. In tetrahydrofolate, one-carbon groups (R, in orange box) are transported on N5 or N10 or both (shown dotted). The points at which methotrexate differs from endogenous folic acid are shown in the blue boxes.

Fig. 56.6 **Simplified diagram of action of methotrexate and fluorouracil on thymidylate synthesis.** Tetrahydrofolate polyglutamate $FH_4(glu)_n$ functions as a carrier of a one-carbon unit, providing the methyl group necessary for the conversion of 2'-deoxyuridylate (DUMP) to 2'-deoxythymidylate (DTMP) by thymidylate synthetase. This one-carbon transfer results in the oxidation of $FH_4(glu)_n$ to $FH_2(glu)_n$. Fluorouracil is converted to FDUMP, which inhibits thymidylate synthetase. DHFR, dihydrofolate reductase.

Fig. 56.7 **The mechanism of action of cytarabine (cytosine arabinoside).** For details of DNA polymerase action, see Figure 50.5. Cytarabine is an analogue of cytosine.

resistance – can lead to nephrotoxicity. This is caused by precipitation of the drug or a metabolite in the renal tubules. High-dose regimens must be followed by 'rescue' with folinic acid (a form of FH_4).

Also chemically related to folate are **raltitrexed**, which inhibits thymidylate synthetase, and **pemetrexed**, which inhibits thymidylate transferase.

Pyrimidine analogues

Fluorouracil, an analogue of uracil, also interferes with DTMP synthesis (Fig. 56.6). It is converted into a 'fraudulent' nucleotide, *fluorodeoxyuridine monophosphate* (FDUMP). This interacts with thymidylate synthetase but cannot be converted into DTMP. The result is inhibition of DNA but not RNA or protein synthesis.

Fluorouracil is usually given parenterally. The main unwanted effects are gastrointestinal epithelial damage and myelotoxicity. Cerebellar disturbances can also occur. Two other drugs, **capecitabine** and **tegafur**, are metabolised to fluorouracil.

Cytarabine (cytosine arabinoside) is an analogue of the naturally occurring nucleoside 2'-deoxycytidine. The drug enters the target cell and undergoes the same phosphorylation reactions as the endogenous nucleoside to give cytosine arabinoside trisphosphate, which inhibits DNA polymerase (see Fig. 56.7). The main unwanted effects are on the bone marrow and the gastrointestinal tract. It also causes nausea and vomiting.

Gemcitabine, an analogue of cytarabine, has fewer unwanted actions, mainly an influenza-like syndrome and mild myelotoxicity. It is often given in combination with other drugs such as cisplatin. **Azacitidine** and **decitabine** inhibit DNA methylase.
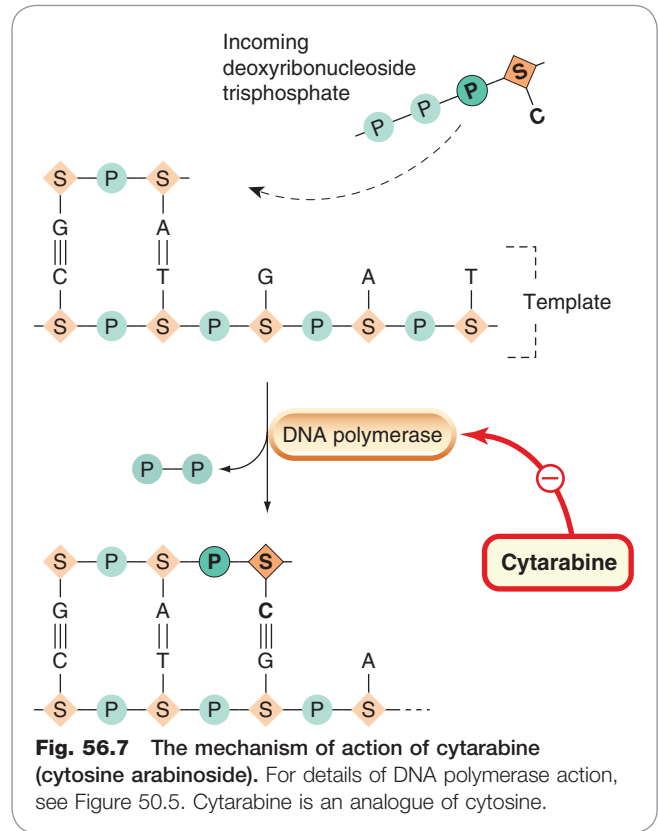
Purine analogues

The main anticancer purine analogues include **cladribine**, **clofarabine**, **fludarabine**, **pentostatin**, **nelarabrine**, **mercaptopurine** and **tioguanine**.

Fludarabine is metabolised to the trisphosphate and inhibits DNA synthesis by actions similar to those of cytarabine. It is myelosuppressive. Pentostatin has a different mechanism of action. It inhibits adenosine deaminase, the enzyme that transforms adenosine to inosine. This action interferes with critical pathways in purine metabolism and can have significant effects on cell proliferation. Cladribine, mercaptopurine and tioguanine are used mainly in the treatment of leukaemia.

Anticancer drugs: antimetabolites

Antimetabolites block or subvert pathways of DNA synthesis.
- *Folate antagonists*. **Methotrexate** inhibits dihydrofolate reductase, preventing generation of tetrahydrofolate interfering with thymidylate synthesis.
- *Pyrimidine analogues*. **Fluorouracil** is converted to a 'fraudulent' nucleotide and inhibits thymidylate synthesis. **Cytarabine** in its trisphosphate form inhibits DNA polymerase. They are potent myelosuppressives.
- *Purine analogues*. **Mercaptopurine** is converted into fraudulent nucleotide. **Fludarabine** in its trisphosphate form inhibits DNA polymerase and is myelosuppressive. **Pentostatin** inhibits adenosine deaminase – a critical pathway in purine metabolism.

CYTOTOXIC ANTIBIOTICS

This is a widely used group of drugs that mainly produce their effects through direct action on DNA. As a rule, they should not be given together with radiotherapy, as the cumulative burden of toxicity is very high.

Doxorubicin and the anthracyclines

Doxorubicin, **idarubicin**, **daunorubicin** and **epirubicin** are widely used anthracycline antibiotics; **mitoxantrone** (**mitozantrone**) is a derivative.

Doxorubicin has several cytotoxic actions. It binds to DNA and inhibits both DNA and RNA synthesis, but its main cytotoxic action appears to be mediated through an effect on topoisomerase II (a DNA gyrase; see Ch. 50), the activity of which is markedly increased in proliferating cells. During replication of the DNA helix, reversible swivelling needs to take place around the replication fork in order to prevent the daughter DNA molecule becoming inextricably entangled during mitotic segregation. The 'swivel' is produced by topoisomerase II, which 'nicks' both DNA strands and subsequently reseals the breaks. Doxorubicin intercalates in the DNA, and its effect is, in essence, to stabilise the DNA–topoisomerase II complex after the strands have been nicked, thus halting the process at this point.

Doxorubicin is given by intravenous infusion. Extravasation at the injection site can cause local necrosis. In addition to the general unwanted effects, the drug can cause cumulative, dose-related cardiac damage, leading to dysrhythmias and heart failure. This action may be the result of generation of free radicals. Marked hair loss frequently occurs.

Dactinomycin

Dactinomycin intercalates in the minor groove of DNA between adjacent guanosine–cytosine pairs, interfering with the movement of RNA polymerase along the gene and thus preventing transcription. There is also evidence that it has a similar action to that of the anthracyclines on topoisomerase II. It produces most of the toxic effects outlined above, except cardiotoxicity. It is mainly used for treating paediatric cancers.

Bleomycins

The bleomycins are a group of metal-chelating glycopeptide antibiotics that degrade preformed DNA, causing chain fragmentation and release of free bases. This action is thought to involve chelation of ferrous iron and interaction with oxygen, resulting in the oxidation of the iron and generation of superoxide and/or hydroxyl radicals. **Bleomycin** is most effective in the G_2 phase of the cell cycle and mitosis, but it is also active against non-dividing cells (i.e. cells in the G_0 phase; Ch. 5, Fig. 5.4). It is often used to treat germline cancer. In contrast to most anticancer drugs, bleomycin causes little myelosuppression: its most serious toxic effect is pulmonary fibrosis, which occurs in 10% of patients treated and is reported to be fatal in 1%. Allergic reactions can also occur. About half the patients manifest mucocutaneous reactions (the palms are frequently affected), and many develop hyperpyrexia.

Mitomycin

Following enzymic activation, **mitomycin** functions as a bifunctional alkylating agent, binding preferentially at O6 of the guanine nucleus. It cross-links DNA and may also degrade DNA through the generation of free radicals. It causes marked delayed myelosuppression and can also cause kidney damage and fibrosis of lung tissue.

> ### Anticancer drugs: cytotoxic antibiotics
>
> - **Doxorubicin** inhibits DNA and RNA synthesis; the DNA effect is mainly through interference with topoisomerase II action. Unwanted effects include nausea, vomiting, myelosuppression and hair loss. It is cardiotoxic in high doses.
> - **Bleomycin** causes fragmentation of DNA chains. It acts on non-dividing cells. Unwanted effects include fever, allergies, mucocutaneous reactions and pulmonary fibrosis. There is virtually no myelosuppression.
> - **Dactinomycin** intercalates in DNA, interfering with RNA polymerase and inhibiting transcription. It also interferes with the action of topoisomerase II. Unwanted effects include nausea, vomiting and myelosuppression.
> - **Mitomycin** is activated to give an alkylating metabolite.

PLANT DERIVATIVES

Several naturally occurring plant products exert potent cytotoxic effects and have a use as anticancer drugs.

Vinca alkaloids

The vinca alkaloids are derived from the Madagascar periwinkle (*Catharanthus roseus*). The principal members of the group are **vincristine**, **vinblastine** and **vindesine**. **Vinflumine**, a fluorinated vinca alkaloid, and **vinorelbine** are semisynthetic vinca alkaloids with similar properties. The drugs bind to tubulin and inhibit its polymerisation into microtubules, preventing spindle formation in dividing cells and causing arrest at metaphase. Their effects become manifest only during mitosis. They also inhibit other cellular activities that require functioning microtubules, such as leukocyte phagocytosis and chemotaxis, as well as axonal transport in neurons.

The adverse effects of vinca alkaloids differ from other anticancer drugs. Vincristine has very mild myelosuppressive activity but is neurotoxic and commonly causes *paraesthesias* (sensory changes), abdominal pain and weakness. Vinblastine is less neurotoxic but causes leukopenia, while vindesine has both moderate myelotoxicity and neurotoxicity. All members of the group can cause reversible hair loss.

Paclitaxel and related compounds

These *taxanes* are derived from a naturally occurring compound found in the bark of the Pacific yew tree (*Taxus* spp.). The group includes **paclitaxel** and the semi-synthetic derivatives **docetaxel** and **cabazitaxel**. These agents act on microtubules, stabilising them (in effect 'freezing' them) in the polymerised state, achieving a similar effect to that of the vinca alkaloids. These drugs are usually given by intravenous infusion. They are generally used to treat breast and lung cancer and paclitaxel,

given with carboplatin, is the treatment of choice for ovarian cancer.

Unwanted effects, which can be serious, include bone marrow suppression and cumulative neurotoxicity. Resistant fluid retention (particularly oedema of the legs) can occur with docetaxel. Hypersensitivity to these compounds is common and requires pretreatment with corticosteroids and antihistamines.

Camptothecins

The camptothecins **irinotecan** and **topotecan**, isolated from the stem of the tree *Camptotheca acuminata*, bind to and inhibit topoisomerase I, high levels of which are present throughout the cell cycle. Diarrhoea and reversible bone marrow depression occur but, in general, these alkaloids have fewer unwanted effects than most other anticancer agents.

Etoposide

Etoposide is derived from mandrake root (*Podophyllum peltatum*). Its mode of action is not clearly known, but it may act by inhibiting mitochondrial function and nucleoside transport, as well as having an effect on topoisomerase II similar to doxorubicin. *Unwanted effects* include nausea and vomiting, myelosuppression and hair loss.

▼ **Compounds from marine sponges. Eribulin** is a naturally occurring compound from marine sponges. Its main inhibitory action on cell division is through inhibition of microtubule function. **Trabectedin**, another compound derived from marine sponges, also disrupts DNA but utilizes a superoxide-related mechanism.

Anticancer drugs: plant derivatives

- **Vincristine** (and related alkaloids) inhibit mitosis at metaphase by binding to tubulin. It is relatively non-toxic but can cause unwanted neuromuscular effects.
- **Etoposide** inhibits DNA synthesis by an action on topoisomerase II and also inhibits mitochondrial function. Common unwanted effects include vomiting, myelosuppression and alopecia.
- **Paclitaxel** (and other taxanes) stabilise microtubules, inhibiting mitosis; it is relatively toxic and hypersensitivity reactions occur.
- **Irinotecan** and **topotecan** inhibit topoisomerase I; They have relatively few toxic effects.

HORMONES

Tumours arising in hormone-sensitive tissues (e.g. breast, uterus, prostate gland) may be *hormone-dependent*, an effect related to the presence of hormone receptors in the malignant cells. Their growth can be inhibited by hormone agonists or antagonists, or by agents that inhibit the synthesis of the hormone.

Hormones or their analogues that have inhibitory actions on target tissues can be used in treatment of tumours of those tissues. Such procedures alone rarely effect a cure but do retard tumour growth and mitigate the symptoms of the cancer, and thus play an important part in the clinical management of sex hormone-dependent tumours.

Glucocorticoids

Glucocorticoids such as **prednisolone** have marked inhibitory effects on lymphocyte proliferation (see Chs 26 and 33) and are used in the treatment of leukaemias and lymphomas. The ability of **dexamethasone** to lower raised intracranial pressure is exploited in treating patients with brain tumours. Glucocorticoids mitigate some of the side effects of anticancer drugs, such as nausea and vomiting, making them useful as supportive therapy when treating other cancers, as well as in palliative care.

Oestrogens

Diethylstilbestrol and **ethinyloestradiol** are still occasionally used in the palliative treatment of androgen-dependent prostatic tumours. These tumours can also be treated with gonadotrophin-releasing hormone analogues (see Ch. 33).

Progestogens

Progestogens such as **megestrol**, **norehisterone** and **medroxyprogesterone** have a role in treatment of endometrial cancer.

Gonadotrophin-releasing hormone analogues

As explained in Chapter 35, analogues of the gonadotrophin-releasing hormones, such as **goserelin**, **buserelin**, **leuprorelin** and **triptorelin**, can, when administered chronically, inhibit gonadotrophin release. These agents are therefore used to treat advanced breast cancer in premenopausal women and prostate cancer. The effect of the transient surge of testosterone secretion that can occur in patients treated in this way for prostate cancer must be prevented by an antiandrogen such as **cyproterone**. **Degaralix** is a gonadotrophin-releasing hormone antagonist used for the treatment of prostate cancer.

Somatostatin analogues

Analogues of somatostatin such as **octreotide** and **lanreotide** (see Ch. 33) are used to relieve the symptoms of neuroendocrine tumours, including hormone-secreting tumours of the gastrointestinal tract such as VIPomas, glucagonomas, carcinoid tumours and gastrinomas. These tumours express somatostatin receptors, activation of which inhibits cell proliferation as well as hormone secretion.

HORMONE ANTAGONISTS

In addition to the hormones themselves, hormone antagonists can also be effective in the treatment of several types of hormone-sensitive tumours.

Antioestrogens

An antioestrogen, **tamoxifen**, is remarkably effective in some cases of hormone-dependent breast cancer and may have a role in preventing these cancers. In breast tissue, tamoxifen competes with endogenous oestrogens for the oestrogen receptors and therefore inhibits the transcription of oestrogen-responsive genes. Tamoxifen is also reported to have cardioprotective effects, partly by virtue of its ability to protect low-density lipoproteins against oxidative damage. Other oestrogen receptor antagonists include **toremifene** and **fulvestrant**.

Unwanted effects are similar to those experienced by women following the menopause. Potentially more serious are hyperplastic events in the endometrium,

which may progress to malignant changes, and the risk of thromboembolism.

Aromatase inhibitors such as **anastrozole**, **letrozole** and **exemestane**, which suppress the synthesis of oestrogen from androgens in the adrenal cortex (but not in the ovary), are also effective in the treatment of breast cancer in postmenopausal (but not in premenopausal) women, in whom they are somewhat more effective than tamoxifen.

Antiandrogens
The androgen antagonists **flutamide**, **cyproterone** and **bicalutamide** may be used either alone or in combination with other agents to treat tumours of the prostate. They are also used to control the testosterone surge ('flare') that is seen when treating patients with gonadorelin analogues. Degarelix does not cause this flare.

Anticancer agents: hormones

Hormones or their antagonists are used in hormone-sensitive tumours:
- **Glucocorticoids** for leukaemias and lymphomas.
- **Tamoxifen** for breast tumours.
- **Gonadotrophin-releasing hormone analogues** for prostate and breast tumours.
- **Antiandrogens** for prostate cancer.
- **Aromatase inhibitors** for postmenopausal breast cancer.

MONOCLONAL ANTIBODIES

Monoclonal antibodies (see Ch. 59) are relatively recent additions to the anticancer armamentarium. In some cases, binding of the antibody to its target activates the host's immune mechanisms and the cancer cell is killed by complement-mediated lysis or by killer T cells (see Ch. 6). Other monoclonal antibodies attach to and inactivate growth factors or their receptors on cancer cells, thus inhibiting the survival pathway and promoting apoptosis (Ch. 5, Fig. 5.5). Unlike most of the cytotoxic drugs described above, they offer the prospect of highly targeted therapy without many of the side effects of conventional chemotherapy. This advantage is offset in most instances as they are often given in combination with more traditional drugs. Several monoclonals are in current clinical use. Their high cost is a significant problem.

Rituximab
Rituximab is a monoclonal antibody that is used (in combination with other chemotherapeutic agents) for treatment of certain types of *lymphoma*. It lyses B lymphocytes by binding to the calcium-channel forming CD20 protein and activating complement. It also sensitises resistant cells to other chemotherapeutic drugs. It is effective in 40–50% of cases when combined with standard chemotherapy.

The drug is given by infusion, and its plasma half-life is approximately 3 days when first given, increasing with each administration to about 8 days by the fourth administration.

Unwanted effects include hypotension, chills and fever during the initial infusions and subsequent hypersensitivity reactions. A cytokine release reaction can occur and has been fatal. The drug may exacerbate cardiovascular disorders.

▼ **Alemtuzumab** is another monoclonal antibody that lyses B lymphocytes, and is used in the treatment of resistant chronic lymphocytic leukaemia. It may also cause a similar cytokine release reaction to that with rituximab. **Ofatumab** is similar. **Brentixumab** additionally targets T cells but in a different manner. It is a conjugate of a cytotoxic drug attached to an antibody that binds to CD30 on malignant cells. It is used to treat *Hodgkin's lymphoma*.

Trastuzumab
Trastuzumab (Herceptin) is a humanised murine monoclonal antibody that binds to an oncogenic protein termed *HER2* (the human epidermal growth factor receptor 2), a member of the wider family of receptors with integral tyrosine kinase activity (Fig. 56.1). There is some evidence that, in addition to inducing host immune responses, trastuzumab induces cell cycle inhibitors p21 and p27 (Ch. 5, Fig. 5.2). Tumour cells, in about 25% of breast cancer patients, overexpress this receptor and the cancer proliferates rapidly. Early results show that trastuzumab given with standard chemotherapy has resulted in a 79% 1-year survival rate in treatment-naive patients with this aggressive form of breast cancer. The drug is often given with a taxane such as docetaxel. Unwanted effects are similar to those with rituximab.

▼ Two mechanistically related compounds are **panitumumab** and **cetuximab**, which bind to epidermal growth factor (EGF) receptors (also overexpressed in a high proportion of tumours). They are used for the treatment of colorectal cancer usually in combination with other agents.

Bevacizumab
Bevacizumab is a humanised monoclonal antibody that is used for the treatment of colorectal cancer but would be expected to be useful for treating other cancers too. It neutralises *VEGF* (vascular endothelial growth factor), thereby preventing the angiogenesis that is crucial to tumour survival. It is administered by intravenous infusion and is generally combined with other agents. A closely related preparation is also given by direct injection into the eye to retard the progress of *acute macular degeneration* (AMD), a common cause of blindness associated with increased retinal vascularisation.

Catumaxomab
Catumaxomab attaches to an epithelial adhesion molecule, EpCAM, which is overexpressed in some malignant cells (e.g. malignant ascites in the peritoneal cavity). The antibody binds to this adhesion molecule and also to T lymphocytes and antigen-presenting cells, thus facilitating the action of the immune system in clearing the cancer.

PROTEIN KINASE INHIBITORS

Imatinib
Hailed as a conceptual breakthrough in targeted chemotherapy, **imatinib** (see Savage & Antman, 2002) is a small-molecule inhibitor of signalling pathway kinases. It inhibits an oncogenic cytoplasmic kinase (Bcr/Abl, see Fig. 56.1. and Fig. 56.8), considered to be a unique factor in the pathogenesis of chronic myeloid leukaemia (CML). It also inhibits platelet-derived growth factor (a receptor
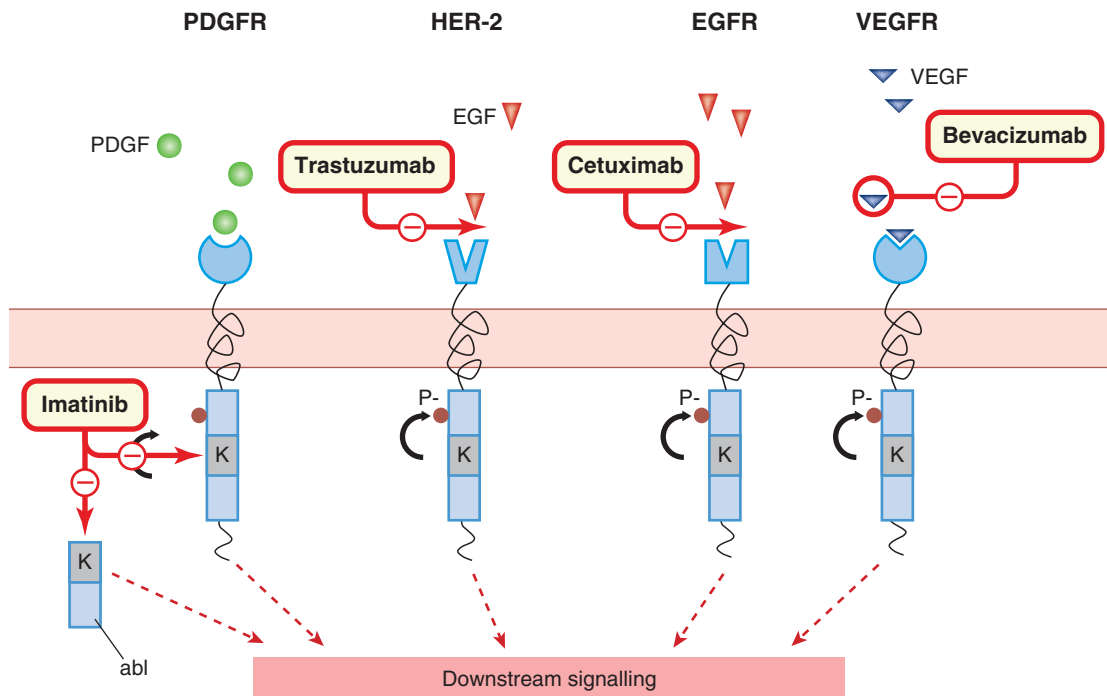
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Fig. 56.8 **The mechanism of action of anticancer monoclonal antibodies and protein kinase inhibitors.** Many tumours overexpress growth factor receptors such as EGFR, the proto-oncogene HER2 or VEGFR. Therapeutic monoclonals can prevent this by interacting directly with the receptor itself (e.g. trastuzumab, cetuximab) or with the ligand (e.g. bevacizumab). An alternate way of reducing this drive on cell proliferation is by inhibiting the downstream signalling cascade. The receptor tyrosine kinases are good targets as are some oncongenic kinases such as bcr/abl. EGFR, epidermal growth factor receptor; HER, human epidermal growth factor; K, kinase domain in receptor; P-, phosphate group; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

tyrosine kinase; Fig. 56.1). It has greatly improved the (hitherto poor) prognosis of patients with CML, and is also used for the treatment of some gastrointestinal tumours not susceptible to surgery.

The drug is given orally. The half-life is about 18 h, and the main site of metabolism is in the liver, where approximately 75% of the drug is converted to a metabolite that is also biologically active. The bulk (81%) of the metabolised drug is excreted in the faeces.

Unwanted effects include gastrointestinal symptoms (pain, diarrhoea, nausea), fatigue, headaches and sometimes rashes. Resistance to imatinib, resulting from mutation of the kinase gene, is a growing problem. It results in little or no cross-resistance to other kinase inhibitors.

▼ Many similar tyrosine kinase inhibitors have recently been developed, including **axitinib**, **crizotinib**, **dastinib**, **erlotinib**, **gefitinib**, **imatinib**, **lapatinib**, **nilotinib**, **pazopanib**, **sunitinib** and **vandentanib**. **Ruxolitinib** inhibits the JAK1 and JAK2 kinases and **vemurafanib** inhibits BRAF kinase. **Sorafenib**, **everolimus** and **temsirolimus** are pan-kinase inhibitors with a similar utility.

MISCELLANEOUS AGENTS

Crisantaspase

▼ **Crisantaspase** is a preparation of the enzyme *asparaginase*, given by injection. It converts asparagine to aspartic acid and ammonia, and is active against tumour cells, such as those of acute lymphoblastic leukaemia, that have lost the capacity to synthesise asparagine and therefore require an exogenous source. As most normal cells are able to synthesise asparagine, the drug has a fairly selective action and has very little suppressive effect on the bone marrow, the mucosa of the gastrointestinal tract or hair follicles. It may cause

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Anticancer drugs: monoclonal antibodies and protein kinase inhibitors

- Many tumours overexpress growth factor receptors that therefore stimulate cell proliferation and tumour growth. This can be inhibited by:
  - monoclonal antibodies, which bind to the extracellular domain of the EGF receptor (e.g. **panitumumab**), the oncogenic receptor HER2 receptor (e.g. **trastuzumab**), or which neutralise the growth factors themselves (e.g. VEGF; **bevacizumab**)
  - protein kinase inhibitors, which prevent downstream signalling triggered by growth factors by inhibiting specific oncogenic kinases (e.g. **imatinib**; bcr/abl) or by inhibiting specific receptor tyrosine kinases (e.g. EGF receptor; **erlotinib**) or several receptor-associated kinases (e.g. **sorefenib**).
- Some monoclonals act directly on lymphocyte cell surface proteins to cause lysis (e.g. **rituximab**), thereby preventing proliferation.

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nausea and vomiting, central nervous system depression, anaphylactic reactions and liver damage.

Hydroxycarbamide

▼ **Hydroxycarbamide** (hydroxyurea) is a urea analogue that inhibits ribonucleotide reductase, thus interfering with the conversion of

ribonucleotides to deoxyribonucleotides. It is mainly used to treat *polycythaemia rubra vera* (a myeloproliferative disorder of the red cell lineage) and (in the past) chronic myelogenous leukaemia. Its use (in somewhat lower dose) in sickle cell anaemia is described in Chapter 25. It has the familiar spectrum of unwanted effects, bone marrow depression being significant.

Bortezomib

▼ **Bortezomib** is a boron-containing tripeptide that inhibits cellular proteasome function. For some reason, rapidly dividing cells are more sensitive than normal cells to this drug, making it a useful anticancer agent. It is mainly used for the treatment of myeloma (a clonal malignancy of plasma cells).

Thalidomide

▼ Investigations of the notorious teratogenic effect of **thalidomide** showed that it has multiple effects on gene transcription, angiogenesis and proteasome function, leading to trials of its efficacy as an anticancer drug. In the event, it proved efficacious in myeloma, for which it is now widely used. The main adverse effect of thalidomide, apart from teratogenesis (irrelevant in myeloma treatment), is peripheral neuropathy, leading to irreversible weakness and sensory loss. It also increases the incidence of thrombosis and stroke.

A thalidomide derivative **lenalidomide** is thought to have fewer adverse effects, but unlike thalidomide, can cause bone marrow depression and neutropenia.

Biological response modifiers and others

▼ Agents that enhance the host's response are referred to as *biological response modifiers*. Some, for example **interferon-α** (and its pegylated derivative), are used in treating some solid tumours and lymphomas, and **aldesleukin** (recombinant interleukin-2) is used in some cases of renal tumours. **Tretinoin** (a form of vitamin A; see Ch. 27) is a powerful inducer of differentiation in leukaemic cells and is used as an adjunct to chemotherapy to induce remission. A related compound is **bexarotene**, a retinoid X receptor antagonist (see Ch. 3) that inhibits cell proliferation and differentiation.

Porfimer and **temoporfin** are haematoporphyrin photosensitizing agents. They accumulate in cells and kill them when excited by the appropriate wavelength light. They are usually used in cases where the light source can be selectively aimed at the tumour (e.g. in the case of obstructing oesophageal tumours).

RESISTANCE TO ANTICANCER DRUGS

The resistance that neoplastic cells manifest to cytotoxic drugs is said to be *primary* (present when the drug is first given) or *acquired* (developing during treatment with the drug). Acquired resistance may result from either *adaptation* of the tumour cells or *mutation*, with the emergence of cells that are less susceptible or resistant to the drug and consequently have a selective advantage over the sensitive cells. The following are examples of various mechanisms of resistance. See Mimeault et al. (2008) for a critical appraisal of this issue.

- *Decreased accumulation of cytotoxic drugs* in cells as a result of the increased expression of cell surface, energy-dependent drug transport proteins. These are responsible for multidrug resistance to many structurally dissimilar anticancer drugs (e.g. doxorubicin, vinblastine and dactinomycin; see Gottesman et al., 2002). An important member of this group is *P-glycoprotein* (P-gp/MDR1; see Ch. 8). P-glycoprotein protects cells against environmental toxins. It functions as a hydrophobic 'vacuum cleaner', picking up foreign chemicals, such as drugs, as they enter the cell membrane and expelling them. Non-cytotoxic agents that reverse

multidrug resistance are being investigated as potential adjuncts to treatment.

- *A decrease in the amount of drug taken up by the cell* (e.g. in the case of methotrexate).
- *Insufficient activation of the drug*. Some drugs require metabolic activation to manifest their antitumour activity. If this fails, they may no longer be effective. Examples include conversion of fluorouracil to FDUMP, phosphorylation of cytarabine and conversion of mercaptopurine to a fraudulent nucleotide.
- *Increase in inactivation* (e.g. cytarabine and mercaptopurine).
- *Increased concentration of target enzyme* (methotrexate).
- *Decreased requirement for substrate* (crisantaspase).
- *Increased utilisation of alternative metabolic pathways* (antimetabolites).
- *Rapid repair of drug-induced DNA damage* (alkylating agents).
- *Altered activity of target*, for example modified topoisomerase II (doxorubicin).
- *Mutations in various genes*, giving rise to resistant target molecules. For example, the *p53* gene and overexpression of the *Bcl-2* gene family (several cytotoxic drugs).

COMBINATION THERAPIES

Treatment with combinations of anticancer agents increases the cytotoxicity against cancer cells without necessarily increasing the general toxicity. For example, methotrexate, which mainly has myelosuppressive toxicity, may be used in a regimen with vincristine, which has mainly neurotoxicity. The few drugs we possess with low myelotoxicity, such as cisplatin and bleomycin, are good candidates for combination regimens. Treatment with combinations of drugs also decreases the possibility of the development of resistance to individual agents. Drugs are often given in large doses intermittently in several courses, with intervals of 2–3 weeks between courses, rather than in small doses continuously, because this permits the bone marrow to regenerate during the intervals. Furthermore, it has been shown that the same total dose of an agent is more effective when given in one or two large doses than in multiple small doses.

CONTROL OF EMESIS AND MYELOSUPPRESSION

EMESIS

The nausea and vomiting induced by many cancer chemotherapy agents are a serious deterrent to patient compliance (see also Ch. 30). It is a particular problem with cisplatin but also complicates therapy with many other compounds, such as the alkylating agents. 5-hydroxytryptamine (HT)$_3$-receptor antagonists such as **ondansetron** or **granisetron** (see Chs 15 and 30) are effective against cytotoxic drug-induced vomiting and have revolutionised cisplatin chemotherapy. Of the other antiemetic agents available, **metoclopramide**, given intravenously in high dose, has proved useful and is often combined with dexamethasone (Ch. 33) or **lorazepam** (Ch. 44), both of which further mitigate the unwanted

effects of chemotherapy. As metoclopramide commonly causes extrapyramidal side effects in children and young adults, **diphenhydramine** (Ch. 26) can be used instead.

MYELOSUPPRESSION

Myelosuppression limits the use of many anticancer agents. Regimens contrived to surmount the problem have included removal of some of the patient's own bone marrow prior to treatment, purging it of cancer cells (using specific monoclonal antibodies) and replacing it after cytotoxic therapy is finished. A protocol in which aliquots of stem cells, harvested from the blood following administration of the growth factor **molgramostim**, which increases their abundance in blood, are expanded *in vitro* using further haemopoietic growth factors (Ch. 25) is now frequently used. The use of such growth factors after replacement of the marrow has been successful in some cases. A further possibility is the introduction, into the extracted bone marrow, of the mutated gene that confers multidrug resistance, so that when replaced, the marrow cells (but not the cancer cells) will be resistant to the cytotoxic action of the anticancer drugs. **Folinic acid** may be given as a supplement to prevent anaemia or as a 'rescue' after high-dose methotrexate.

FUTURE DEVELOPMENTS

As the reader will have judged by now, our current approach to cancer chemotherapy embraces an eclectic mixture of drugs – some very old and some very new – in an attempt to target selectively cancer cells. Real therapeutic progress has been achieved, although 'cancer' as a disease (actually many different diseases with a similar outcome) has not been comprehensively defeated and remains a massive challenge for future generations of researchers. In this therapeutic area, probably more than in any other, the debate about the risk–benefit of treatment and the patient quality of life issues has taken centre stage and remains a major area of concern (see Duric & Stockler, 2001; Klastersky & Paesmans, 2001).

Of the recent advances in drug therapy, the tyrosine kinase inhibitors and the biologics have arguably been the most innovative advances. Further drugs of the kinase inhibitor type are under active investigation (see Vargas et al., 2013), as are anti-angiogenic drugs (similar to bevacizumab; see Ferrarotto & Hoff, 2013). Novel drugs targeting HER2-receptor in breast cancer have been reviewed by Abramson and Arteaga (2011). Warner and Gustafsson (2010) have highlighted the opportunities afforded by the discovery of a further isoform of the oestrogen receptor for the treatment of hormone-dependent breast and other cancers.

▼ For years, epidemiological and experimental evidence has been accumulating, which suggests that chronic use of cyclo-oxygenase (COX) inhibitors (see Ch. 26) protect against cancer of the gastrointestinal tract and possibly other sites as well. The COX-2 isoform is overexpressed in about 85% of cancers, and prostanoids originating from this source may activate signalling pathways that enable cells to escape from apoptotic death. The literature has been controversial but the balance of evidence now favours the notion that COX-2 may be a potentially important target for anticancer drug development (see Khan et al., 2011). COX-2 inhibitors could therefore be useful in the treatment of some cancers, either alone or in combination with conventional chemotherapeutic agents (Ghosh et al., 2010; Kraus et al., 2013). Ironically, some authors (Gurpinar et al., 2013) argue that the mechanism of action of these inhibitors in cancer models is unrelated to COX inhibition. No doubt these apparent paradoxes will be resolved with the passage of time.

Much work is going into genotyping of tumour tissue as a guide to selecting the best drug combination to use in treating an individual patient, based on the particular genetic abnormality present in the tumour cells (see Patel et al., 2013 for a short review). This approach, still in its early stages, is beginning to yield promising approaches to optimising treatment of melanoma and lung cancer, and is expected to develop rapidly.

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Useful Web resources

(*The US equivalent of the website below. The best sections for you are those marked* Health Information Seekers *and* Professionals)

<www.cancerresearchuk.org> (*The website of Cancer Research UK, the largest cancer charity in the UK. Contains valuable data on the epidemiology and treatment of cancer, including links to clinical trials. An excellent resource*)

57

Harmful effects of drugs

OVERVIEW

This chapter addresses harmful effects of drugs, both in the context of therapeutic use – so-called adverse drug reactions – and of deliberate or accidental overdose. We are concerned here with serious harm, sometimes life-threatening or irreversible, distinct from the minor side effects that virtually all drugs produce, as described throughout this book. The classification of adverse drug reactions is considered, followed by aspects of drug toxicity, namely toxicity testing in drug development, mechanisms of toxin-induced cell damage, mutagenesis and carcinogenicity, teratogenesis and allergic reactions.

INTRODUCTION

Paracelsus, a 16th-century alchemist, is credited with the aphorism that all drugs are poisons: '… the dosage makes it either a poison or a remedy'. Today, toxic effects of drugs remain clinically important in the context of overdose (self-poisoning accounts for approximately 10% of the workload of emergency medicine departments in the UK; by contrast, homicidal poisoning is extremely uncommon). Some susceptible individuals may experience dose-related toxicity even during therapeutic dosing; some of this susceptibility is genetically determined, and genomic testing as a means of avoiding such harms is beginning to make its way into the clinic (Ch. 11).

Rigorous toxicity testing in animals (see p. 693), including tests for carcinogenicity, teratogenicity and organ-specific toxicities, is carried out on potential new drugs during development (see Ch. 60), often leading to abandonment of the compound before it is tested in humans. These toxicity studies form part of the package of information routinely submitted to regulatory agencies by drug companies seeking approval to market a new drug. Nevertheless, harmful effects are often encountered after a drug is marketed for human use, due to the emergence of adverse effects not detected in animals. These harms are usually referred to as 'adverse drug reactions' (ADRs) and are of great concern to drug regulatory authorities, which are charged with establishing the safety as well as the efficacy of drugs. Unpredictable events are of particular concern. Some ADRs are predictable as a consequence of the main pharmacological effect of the drug and are relatively easily recognised, but some (e.g. immunological reactions), are unpredictable, sometimes serious, and likely to occur only in some patients.

Clinically important ADRs are common, costly and avoidable (see Pirmohamed et al., 2004).[1] Any organ can

[1]6.5% of hospital admissions were due to ADRs at a projected annual cost of £466 million in the UK. Antiplatelet drugs, diuretics, non-steroidal anti-inflammatory drugs and anticoagulants between them accounted for 50% of the ADRs. 2.3% of the patients died. Most events were avoidable.

be the principal target, and several organ systems can be involved simultaneously. The symptoms and signs sometimes closely shadow drug administration and discontinuation, but in other cases adverse effects only occur during prolonged use (*osteoporosis* during continued high-dose glucocorticoid therapy [Ch. 33], or *tardive dyskinesia* during continuous use of antipsychotic drugs [Ch. 46], for example). Some adverse effects occur on ending treatment, either within a few days (e.g. tachycardia on abrupt discontinuation of β-adrenoceptor blockade) or after a delay, first appearing months or years after treatment is discontinued, as in the case of some second malignancies following successful chemotherapy. Consequently, anticipating, avoiding, recognising and responding to adverse drug reactions are among the most challenging and important parts of clinical practice.

CLASSIFICATION OF ADVERSE DRUG REACTIONS

Harmful effects of drugs may or may not be related to the known mechanism of action of the drug In either case, individual variation (see Ch. 11) is a major factor in determining the response of a particular patient and their susceptibility to harm. Aronson & Ferner (2003) have suggested that ADRs be described according to the **do**se, **t**ime course and **s**usceptibility (DoTS).

ADVERSE EFFECTS RELATED TO THE KNOWN PHARMACOLOGICAL ACTION OF THE DRUG

Many adverse effects related to the known pharmacological actions of the drug are predictable, at least if these actions are well understood. They are sometimes referred to as type A ('augmented') adverse reactions (Rawlins & Thomson, 1985) and are related to dose and individual susceptibility. Many such reactions have been described in previous chapters. For example, postural hypotension occurs with α_1-adrenoceptor antagonists, bleeding with anticoagulants, sedation with anxiolytics and so on. In many instances, this type of unwanted effect is reversible, and the problem can often be dealt with by reducing the dose. Such effects are sometimes serious (e.g. intracerebral bleeding caused by anticoagulants, hypoglycaemic coma from insulin), and occasionally they are not easily reversible, for example drug dependence produced by opioid analgesics (see Ch. 49).

Some adverse effects related to the main action of a drug result in discrete events rather than graded symptoms, and can be difficult to detect. For example, drugs that block cyclo-oxygenase (COX)-2 (including 'coxibs', for example **rofecoxib**, **celecoxib**, **valdecoxib**, as well as some conventional non-steroidal anti-inflammatory drugs, NSAIDs) increase the risk of myocardial infarction in a dose-dependent manner (Ch. 26). This potential was predictable from the ability of these drugs to inhibit

prostacyclin biosynthesis and increase arterial blood pressure, and early studies gave a hint of such problems. The effect was difficult to prove because of the high background incidence of coronary thrombosis, and it was only when placebo-controlled trials were performed for another indication (in the hope that COX-2 inhibitors could prevent bowel cancer) that this effect was confirmed unequivocally.

ADVERSE EFFECTS UNRELATED TO THE KNOWN PHARMACOLOGICAL ACTION OF THE DRUG

Adverse effects unrelated to the main pharmacological effect may be predictable when a drug is taken in excessive dose, for example **paracetamol** hepatotoxicity (see below) or **aspirin**-induced tinnitus; or when susceptibility is increased, for example during pregnancy or by a predisposing disorder such as glucose 6-phosphate dehydrogenase deficiency or a mutation in the mitochondrial DNA that predisposes to aminoglycoside ototoxicity (Ch. 11).

Unpredictable reactions unrelated to the main effect of the drug (sometimes termed *idiosyncratic reactions*, or type B for Bizarre in the Rawlins & Thomson (1985) classification) are often initiated by a chemically reactive metabolite rather than the parent drug. Examples of such ADRs, which are often immunological in nature, include drug-induced hepatic or renal necrosis, bone marrow suppression, carcinogenesis and disordered fetal development. Uncommon but severe unpredictable adverse effects that have been mentioned in earlier chapters include aplastic anaemia from **chloramphenicol** and anaphylaxis in response to **penicillin**. They are usually severe – otherwise they would go unrecognised – and their existence is important in establishing the safety of medicines.

DRUG TOXICITY

TOXICITY TESTING

Toxicity testing in animals is carried out on new drugs to identify potential hazards before administering them to humans. It involves the use of a wide range of tests in different species, with long-term administration of the drug, regular monitoring for physiological or biochemical abnormalities, and a detailed postmortem examination at the end of the trial to detect any gross or histological abnormalities. Toxicity testing is performed with doses well above the expected therapeutic range, and establishes which tissues or organs are likely 'targets' of toxic effects of the drug. Recovery studies are performed to assess whether toxic effects are reversible, and particular attention is paid to irreversible changes such as carcinogenesis or neurodegeneration. The basic premise is that toxic effects caused by a drug are similar in humans and other animals. There are, however, wide interspecies variations, especially in metabolising enzymes; consequently, a toxic metabolite formed in one species may not be formed in another, and so toxicity testing in animals is not always a reliable guide. **Pronethalol**, the first β-adrenoceptor antagonist synthesised, was not developed because it caused carcinogenicity in mice; it subsequently emerged that carcinogenicity occurred only in the one strain tested – but by then other β blockers were already in development.

Toxic effects can range from negligible to so severe as to preclude further development of the compound. Intermediate levels of toxicity are more acceptable in drugs intended for severe illnesses (e.g. AIDS or cancers), and decisions on whether or not to continue development are often difficult. If development does proceed, safety monitoring can be concentrated on the system 'flagged' as a potential target of toxicity by the animal studies.[2] *Safety* of a drug (as distinct from toxicity) can be established only during use in humans.

> **Types of drug toxicity**
>
> - Toxic effects of drugs can be:
>   - related to the principal pharmacological action (e.g. bleeding with anticoagulants)
>   - unrelated to the principal pharmacological action (e.g. liver damage with **paracetamol**).
> - Some adverse reactions that occur with ordinary therapeutic dosage are initially unpredictable, serious and uncommon (e.g. agranulocytosis with **carbimazole**). Such reactions (termed idiosyncratic) are almost inevitably detected only after widespread use of a new drug. It is sometimes possible to develop a test to exclude susceptible subjects from drug exposure (e.g. mitochondrial DNA variants/increased susceptibility to aminoglycoside ototoxicity).
> - Adverse effects unrelated to the main action of a drug are often caused by reactive metabolites and/or immunological reactions.

GENERAL MECHANISMS OF TOXIN-INDUCED CELL DAMAGE AND CELL DEATH

Toxic concentrations of drugs or drug metabolites can cause necrosis; however, programmed cell death (apoptosis; see Ch. 5) is increasingly recognised to be of equal or greater importance, especially in chronic toxicity.

Chemically reactive drug metabolites can form covalent bonds with target molecules, or can damage tissue by non-covalent mechanisms. The liver is of great importance in drug metabolism (Ch. 9), and hepatocytes are exposed to high concentrations of nascent metabolites. Drugs and their polar metabolites are concentrated in renal tubular fluid as water is reabsorbed, so renal tubules are exposed to higher concentrations than are other tissues. Several hepatotoxic drugs (e.g. paracetamol) are also nephrotoxic. Consequently, hepatic or renal damage are common reasons for abandoning development of drugs during toxicity testing and chemical pathology tests of hepatic damage (usually levels of transaminase

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[2]The value of toxicity testing is illustrated by experience with **triparanol**, a cholesterol-lowering drug marketed in the USA in 1959. Three years later, a team from the Food and Drug Administration, acting on a tip-off, paid the manufacturer a surprise visit that revealed falsification of toxicology data demonstrating cataracts in rats and dogs. The drug was withdrawn, but some patients who had been taking it for a year or more did develop cataracts. Regulatory authorities now require that toxicity testing is performed under a tightly defined code of practice (Good Laboratory Practice), which incorporates many safeguards to minimise the risk of error or fraud.

enzymes measured in blood plasma or serum) and renal function (usually creatinine concentration) are routine.

NON-COVALENT INTERACTIONS

▼ Reactive metabolites of drugs are implicated in several potentially cytotoxic, non-covalent processes, including:

* lipid peroxidation
* generation of toxic reactive oxygen species
* depletion of reduced glutathione (GSH)
* modification of sulfhydryl groups.

Lipid peroxidation

▼ Peroxidation of unsaturated lipids can be initiated either by reactive metabolites or by reactive oxygen species (see Fig. 57.1). Lipid peroxyradicals (ROO$^\bullet$) can produce lipid hydroperoxides (ROOH), which produce further lipid peroxyradicals. This chain reaction – a peroxidative cascade – may eventually affect much of the membrane lipid. Defence mechanisms, for example GSH peroxidase and vitamin E, protect against this. Cell damage results from alteration of membrane permeability or from reactions of the products of lipid peroxidation with proteins.

Reactive oxygen species

▼ Reduction of molecular oxygen to superoxide anion ($O_2^{-\bullet}$) may be followed by enzymic conversion to hydrogen peroxide (H_2O_2), hydroperoxy (HOO$^\bullet$) and hydroxyl (OH$^\bullet$) radicals or singlet oxygen. These reactive oxygen species are cytotoxic, both directly and through lipid peroxidation, and are important in excitotoxicity and neurodegeneration (Ch. 40, Fig. 40.2).

Depletion of glutathione

▼ The GSH redox cycle protects cells from oxidative stress. GSH can be depleted by accumulation of normal oxidative products of cell metabolism, or by the action of toxic chemicals. GSH is normally maintained in a redox couple with its disulfide, GSSG. Oxidising species convert GSH to GSSG, GSH being regenerated by NADPH-dependent GSSG reductase. When cellular GSH falls to about 20–30% of normal, cellular defence against toxic compounds is impaired and cell death can result.

Modification of sulfhydryl groups

▼ Modification of sulfhydryl groups can be produced either by oxidising species that alter sulfhydryl groups reversibly or by covalent interaction. Free sulfhydryl groups have a critical role in the catalytic activity of many enzymes. Important targets for sulfhydryl modification by reactive metabolites include the cytoskeletal protein actin GSH reductase and Ca^{2+}-transporting ATPases in the plasma membrane and endoplasmic reticulum. These maintain cytoplasmic Ca^{2+} concentration at approximately 0.1 μmol/l in the face of an extracellular Ca^{2+} concentration of more than 1 mmol/l. A sustained rise in cell Ca^{2+} occurs with inactivation of these enzymes (or with increased membrane permeability; see above), and this compromises cell viability. Lethal processes leading to cell death after acute Ca^{2+} overload include activation of degradative enzymes (neutral proteases, phospholipases, endonucleases) and protein kinases, mitochondrial damage and cytoskeletal alterations (e.g. modification of association between actin and actin-binding proteins).

COVALENT INTERACTIONS

Targets for covalent interactions include DNA, proteins/peptides, lipids and carbohydrates. Covalent bonding to DNA is a basic mechanism of mutagenic chemicals; this is dealt with below. Several non-mutagenic chemicals also form covalent bonds with macromolecules, but the relationship between this and cell damage is incompletely understood. For example, the cholinesterase inhibitor paraoxon (the active metabolite of the insecticide parathion) binds acetylcholinesterase at the neuromuscular junction (Ch. 13) and causes necrosis of skeletal muscle.

One toxin from an exceptionally poisonous toadstool, *Amanita phalloides*, binds actin, and another binds RNA polymerase, interfering with actin depolymerisation and protein synthesis, respectively.

General mechanisms of cell damage and cell death

* Drug-induced cell damage/death is usually caused by reactive metabolites of the drug, involving non-covalent and/or covalent interactions with target molecules. Cell death often occurs by apoptosis.
* Non-covalent interactions include:
  – lipid peroxidation via a chain reaction
  – generation of cytotoxic reactive oxygen species
  – depletion of reduced glutathione
  – modification of sulfhydryl groups on key enzymes (e.g. Ca^{2+}-ATPase) and structural proteins.
* Covalent interactions, for example adduct formation between a metabolite of **paracetamol** (NAPBQI: *N*-acetyl-*p*-benzoquinone imine) and cellular macromolecules (Fig. 57.1). Covalent binding to protein can produce an immunogen; binding to DNA can cause carcinogenesis and teratogenesis.

HEPATOTOXICITY

Many therapeutic drugs cause liver damage, manifested clinically as hepatitis or (in less severe cases) only as laboratory abnormalities (e.g. increased activity of plasma aspartate transaminase, an enzyme released from damaged liver cells). Paracetamol and **halothane** cause hepatotoxicity by the mechanisms of cell damage outlined above. Genetic differences in drug metabolism (see Ch. 11) have been implicated in some instances (e.g. **isoniazid**, **phenytoin**). Mild drug-induced abnormalities of liver function are not uncommon, but the mechanism of liver injury is often uncertain (e.g. *statins*; Ch. 23). It is not always necessary to discontinue a drug when such mild laboratory abnormalities occur, but the occurrence of cirrhosis as a result of long-term low-dose **methotrexate** treatment for arthritis or psoriasis (see Chs 26 and 27) argues for caution. Hepatotoxicity of a different kind, namely reversible obstructive jaundice, occurs with **chlorpromazine** (Ch. 46) and androgens (Ch. 35).

Hepatotoxicity caused by **paracetamol** overdose remains a common cause of death following self-poisoning. An outline is given in Chapter 26. Paracetamol poisoning exemplifies many of the general mechanisms of cell damage outlined above. With toxic doses of paracetamol, the enzymes catalysing the normal conjugation reactions are saturated, and mixed-function oxidases instead convert the drug to the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPBQI). As explained in Chapter 9, paracetamol toxicity is increased in patients in whom P450 enzymes have been induced, for instance by chronic excessive consumption of alcohol. NAPBQI initiates several of the covalent and non-covalent interactions described above and illustrated in Figure 57.1. Oxidative stress from GSH depletion is important in leading
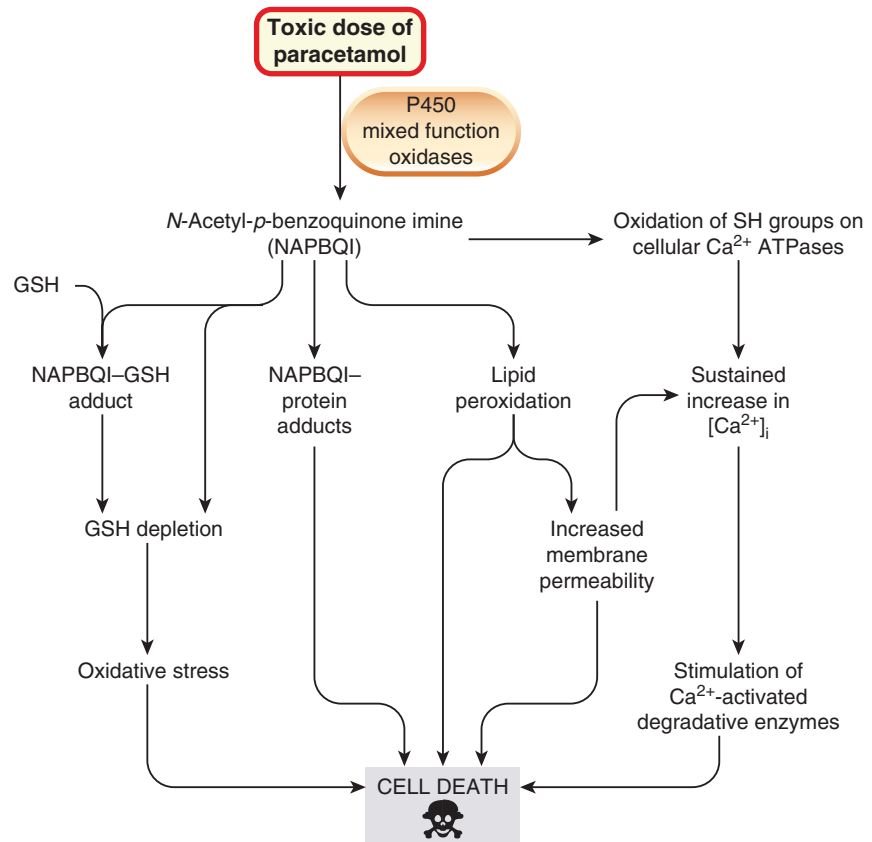
Fig. 57.1 Potential mechanisms of liver cell death resulting from the metabolism of paracetamol to *N*-acetyl-*p*-benzoquinone imine (NAPBQI). GSH, glutathione. *(Based on data from Boobis AR et al. 1989 Trends Pharmacol Sci 10, 275–280 and Nelson SD, Pearson PG 1990 Annu Rev Pharmacol Toxicol 30, 169.)*

to cell death. Regeneration of GSH from GSSG depends on the availability of cysteine, the intracellular availability of which can be limiting. *Acetylcysteine* or *methionine* can substitute for cysteine, increasing GSH availability; they are used to treat patients with paracetamol poisoning.

Liver damage can also be produced by immunological mechanisms (see p. 701), which have been particularly implicated in halothane hepatitis (see Ch. 41).

Hepatotoxicity

- Hepatocytes are exposed to reactive metabolites of drugs as these are formed by P450 enzymes.
- Liver damage is produced by several mechanisms of cell injury; **paracetamol** exemplifies many of these (see Fig. 57.1).
- Some drugs (e.g. **chlorpromazine**) can cause reversible cholestatic jaundice.
- Immunological mechanisms are sometimes implicated (e.g. **halothane**).

Table 57.1 Adverse effects of non-steroidal anti-inflammatory drugs on the kidney

Cause	Adverse effects
Principal pharmacological action (i.e. inhibition of prostaglandin biosynthesis)	Acute ischaemic renal failure Sodium retention (leading to or exacerbating hypertension and/or heart failure) Water retention Hyporeninaemic hypoaldosteronism (leading to hyperkalaemia)
Unrelated to principal pharmacological action (allergic-type interstitial nephritis)	Renal failure Proteinuria
Unknown whether or not related to principal pharmacological action (analgesic nephropathy)	Papillary necrosis Chronic renal failure

Adapted from Murray & Brater 1993.

NEPHROTOXICITY

Drug-induced nephrotoxicity is a common clinical problem: NSAIDs (Table 57.1) and angiotensin-converting enzyme (ACE) inhibitors are among the commonest precipitants of acute renal failure. This is usually caused by the principal pharmacological actions of these drugs, which, although well tolerated in healthy people, cause renal failure in patients with diseases that jeopardise glomerular filtration.

Nephrotoxicity

- Renal tubular cells are exposed to high concentrations of drugs and metabolites as urine is concentrated.
- Renal damage can cause papillary and/or tubular necrosis.
- Inhibition of prostaglandin synthesis by non-steroidal anti-inflammatory drugs causes vasoconstriction and lowers glomerular filtration rate.

MUTAGENESIS AND ASSESSMENT OF GENOTOXIC POTENTIAL

Drug-induced mutagenesis is one important cause of carcinogenesis and of teratogenesis. Registration of pharmaceuticals requires a comprehensive assessment of their genotoxic potential. Because no single test is adequate, the usual approach is to carry out a battery of *in vitro* and *in vivo* tests for genotoxicity, usually comprising tests for gene mutation in bacteria, *in vitro* and *in vivo* tests for chromosome damage, and *in vivo* tests for reproductive toxicity and carcinogenicity (see below).

BIOCHEMICAL MECHANISMS OF MUTAGENESIS

Chemical agents cause mutation by covalent modification of DNA. Certain mutations result in carcinogenesis, because the affected DNA sequence codes for a protein that regulates cell growth. It usually requires more than one mutation in a cell to initiate the changes that result in malignancy, mutations in proto-oncogenes (which regulate cell growth) and tumour suppressor genes (which code for products that inhibit the transcription of oncogenes) being particularly implicated (see Chs 5 and 56).

▼ Most chemical carcinogens act by modifying bases in DNA, particularly guanine, the O6 and N7 positions of which readily combine covalently with reactive metabolites of chemical carcinogens. Substitution at the O6 position is the more likely to produce a permanent mutagenic effect, because N7 substitutions are usually quickly repaired.

The accessibility of bases in DNA to chemical attack is greatest when DNA is in the process of replication (i.e. during cell division). The likelihood of genetic damage by many mutagens is therefore related to the frequency of cell division. The developing fetus is particularly susceptible, and mutagens are also potentially teratogenic for this reason (see p. 698). This is also important in relation to mutagenesis of germ cells, particularly in girls, because in humans the production of primary oocytes occurs by a rapid succession of mitotic divisions very early in embryogenesis. Each primary oocyte then undergoes only two further divisions much later in life, at the time of ovulation. It is consequently during early pregnancy that germ cells of the developing female embryo are most likely to undergo mutagenesis, the mutations being transmitted to progeny conceived many years later. In the male, germ cell divisions occur throughout life, and sensitivity of germ cells to mutagens is continuously present.

CARCINOGENESIS

Alteration of DNA is the first step in carcinogenesis (see Chs 5 and 56). Carcinogenic compounds can interact directly with DNA (genotoxic carcinogens) or act at a later stage to increase the likelihood that mutation will result in a tumour (epigenetic carcinogens; Fig. 57.2).
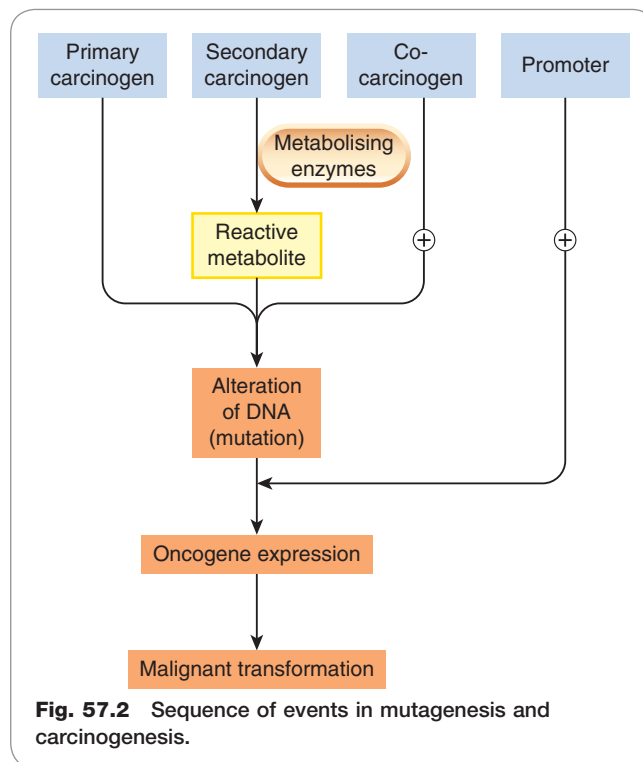
Fig. 57.2 Sequence of events in mutagenesis and carcinogenesis.

Mutagenesis and carcinogenicity

- Mutagenesis involves modification of DNA.
- Mutation of proto-oncogenes or tumour suppressor genes leads to carcinogenesis. More than one mutation is usually required.
- Drugs are relatively uncommon (but not unimportant) causes of birth defects and cancers.

MEASUREMENT OF MUTAGENICITY AND CARCINOGENICITY

Much effort has gone into developing assays to detect mutagenicity and carcinogenicity. *In vitro* tests for *mutagenicity* are used to screen large numbers of compounds but are unreliable as predictors of carcinogenicity. Whole-animal tests for carcinogenicity are expensive and time-consuming but are usually required by regulatory authorities before a new drug is licensed for use in humans. The main limitation of this kind of study is that there are important species differences, mainly to do with the metabolism of the foreign compound and the formation of reactive products.

The widely used *Ames test* for mutagenicity measures the effect of substances on the rate of back-mutation (i.e. reversion from mutant to wild-type form) in *Salmonella typhimurium*.

▼ The wild-type strain can grow in a medium containing no added amino acids, because it can synthesise all the amino acids it needs. A mutant form of the organism cannot make histidine in this way and therefore grows only on a medium containing this amino acid. The Ames test involves growing the mutant form on a medium containing a small amount of histidine, plus the drug to be tested. After several divisions, the histidine becomes depleted, and the only

cells that continue dividing are those that have back-mutated to the wild type. A count of colonies following subculture on plates deficient in histidine gives a measure of the mutation rate.

Primary carcinogens cause mutation by a direct action on bacterial DNA, but most carcinogens have to be converted to an active metabolite (see Fig. 57.2). Therefore it is necessary to include, in the culture, enzymes that catalyse the necessary conversion. An extract of liver from a rat treated with **phenobarbital** to induce liver enzymes is usually employed. There are many variations based on the same principle.

Other short-term *in vitro* tests for genotoxic chemicals include measurements of mutagenesis in mouse lymphoma cells, and assays for chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells. However, all the *in vitro* tests give some false-positive and some false-negative results.

In vivo tests for carcinogenicity entail detection of tumours in groups of test animals. Carcinogenicity tests are inevitably slow, because there is usually a latency of months or years before tumours develop. Furthermore, tumours can develop spontaneously in control animals, and the results often provide only equivocal evidence of carcinogenicity of the test drug, making it difficult for industry and regulatory authorities to decide on further development and possible licensing of a product. None of the tests so far described can reliably detect epigenetic carcinogens. To do this, tests that measure the effect of the

substance on tumour formation in the presence of a threshold dose of a separate genotoxic agents are being evaluated.

Few therapeutic drugs in clinical use are known to increase the risk of cancer, the most important groups being drugs that act on DNA, i.e. cytotoxic and immunosuppressant drugs (Chs 56 and 26, respectively), and sex hormones (e.g. *oestrogens*, Ch. 35).

TERATOGENESIS AND DRUG-INDUCED FETAL DAMAGE

Teratogenesis signifies the production of gross structural malformations during fetal development, in distinction from other kinds of drug-induced fetal damage such as growth retardation, dysplasia (e.g. iodide-associated goitre) or the asymmetrical limb reduction resulting from vasoconstriction caused by **cocaine** (see Ch. 49) in an otherwise normally developing limb. Examples of drugs that affect fetal development adversely are given in Table 57.2.

The importance of X irradiation and rubella infection as causes of fetal malformation was recognised early in the 20th century, but it was not until 1960 that drugs were implicated as causative agents in teratogenesis: the shocking experience with **thalidomide** led to a widespread reappraisal of many other drugs in clinical use, and to the setting up of drug regulatory bodies in many countries. Most birth

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Table 57.2   Some drugs reported to have adverse effects on human fetal development

Agent	Effect(s)	Teratogenicity[a]	See chapter
Thalidomide	Phocomelia, heart defects, gut atresia, etc.	K	This chapter
Penicillamine	Loose skin etc.	K	26
Warfarin	Saddle nose; retarded growth; defects of limbs, eyes, central nervous system	K	24
Corticosteroids	Cleft palate and congenital cataract – rare	–	33
Androgens	Masculinisation in female	–	35
Oestrogens	Testicular atrophy in male	–	35
Stilbestrol	Vaginal adenosis in female fetus, also vaginal or cervical cancer	20+ years later	35
Phenytoin	Cleft lip/palate, microcephaly, mental retardation	K	45
Valproate	Neural tube defects (e.g. spina bifida)	K	45
Carbamazepine	Retardation of fetal head growth	S	45
Cytotoxic drugs (especially folate antagonists)	Hydrocephalus, cleft palate, neural tube defects, etc.	K	56
Aminoglycosides	Deafness	–	51
Tetracycline	Staining of bones and teeth, thin tooth enamel, impaired bone growth	S	51
Ethanol	Fetal alcohol syndrome	K	49
Retinoids	Hydrocephalus etc.	K	27
Angiotensin-converting enzyme inhibitors	Oligohydramnios, renal failure	K	22

[a]K, known teratogen (in experimental animals and/or humans); S, suspected teratogen (in experimental animals and/or humans).
Adapted from Juchau MR 1989 Bioactivation in chemical teratogenesis. Ann Rev Pharmacol Toxicol 29, 165.

Carcinogens

- Carcinogens can be:
  - genotoxic, i.e. causing mutations directly (primary carcinogens) or after conversion to reactive metabolites (secondary carcinogens)
  - epigenetic, i.e. increasing the possibility that a mutagen will cause cancer, although not themselves mutagenic.
- New drugs are tested for mutagenicity and carcinogenicity.
- The Ames test for mutagenicity measures back-mutation, in histidine-free medium, of a mutant *Salmonella typhimurium* (which, unlike the wild-type, cannot grow without histidine) in the presence of:
  - the chemical to be tested
  - a liver microsomal enzyme preparation for generating reactive metabolites.
- Colony growth indicates that mutagenesis has occurred. The test is rapid and inexpensive, but some false-positives and false-negatives occur.
- Carcinogenicity testing:
  - involves chronic dosing of groups of animals
  - is expensive and time-consuming
  - does not readily detect epigenetic carcinogens.

defects (about 70%) occur with no recognisable causative factor. Drug or chemical exposure during pregnancy is estimated to account for only approximately 1% of all fetal malformations. Fetal malformations are common, so the absolute numbers of children affected are substantial.

MECHANISM OF TERATOGENESIS

The timing of the teratogenic insult in relation to fetal development is critical in determining the type and extent of damage. Mammalian fetal development passes through three phases (Table 57.3):

1. blastocyst formation
2. organogenesis
3. histogenesis and maturation of function.

Cell division is the main process occurring during blasto-cyst formation. During this phase, drugs can kill the embryo by inhibiting cell division, but provided the embryo survives, its subsequent development does not

generally seem to be compromised. Ethanol is an exception, affecting development even at this very early stage (Ch. 49).

Drugs can cause gross malformations if administered during organogenesis (days 17–60 in humans). The structural organisation of the embryo occurs in a well-defined sequence: eye and brain, skeleton and limbs, heart and major vessels, palate, genitourinary system. The type of malformation produced thus depends on the time of exposure to the teratogen.

The cellular mechanisms by which teratogenic substances produce their effects are not at all well understood. There is a considerable overlap between mutagenicity and teratogenicity. In one large survey, among 78 compounds, 34 were both teratogenic and mutagenic, 19 were negative in both tests and 25 (among them thalidomide) were positive in one but not the other. Damage to DNA is important but not the only factor. The control of morphogenesis is poorly understood; vitamin A derivatives (retinoids) are involved and are potent teratogens (see p. 699 and Ch. 27). Known teratogens also include several drugs (e.g. **methotrexate** and **phenytoin**) that do not react directly with DNA but which inhibit its synthesis by their effects on folate metabolism (see Ch. 25). Administration of **folate** during pregnancy reduces the frequency of both spontaneous and drug-induced malformations, especially neural tube defects.

The fetus depends on an adequate supply of nutrients during the final stage of histogenesis and functional maturation, and development is regulated by a variety of hormones. Gross structural malformations do not arise from exposure to mutagens at this stage, but drugs that interfere with the supply of nutrients or with the hormonal milieu may have deleterious effects on growth and development. Exposure of a female fetus to androgens at this stage can cause masculinisation. **Stilbestrol** (a synthetic estrogen, now seldom used, licensed to treat breast or prostate cancer) was commonly given to pregnant women with a history of recurrent miscarriage during the 1950s (for unsound reasons). Used in this way it caused dysplasia of the vagina of female infants and an increased incidence of carcinoma of the vagina, a rare malignancy with almost no background incidence, in such offspring in their teens and twenties. Angiotensin II plays an important part in the later stages of fetal development and in renal function in the fetus, and ACE inhibitors and angiotensin receptor antagonists (Ch. 22) cause oligo-hydramnios and renal failure if administered during later stages of pregnancy, and fetal malformations if given earlier.

Table 57.3   The nature of drug effects on fetal development

Stage	Gestation period in humans	Main cellular process(es)	Affected by
Blastocyst formation	0–16 days	Cell division	Cytotoxic drugs, ?alcohol
Organogenesis	17–60 days approximately	Division Migration Differentiation Death	Teratogens Teratogens Teratogens Teratogens
Histogenesis and functional maturation	60 days to term	As above	Miscellaneous drugs (e.g. alcohol, nicotine, antithyroid drugs, steroids)

TESTING FOR TERATOGENICITY

The thalidomide disaster dramatically brought home the need for teratogenicity studies on new therapeutic drugs. Detection of drug-induced teratogenesis in humans is a particularly difficult problem because the 'spontaneous' malformation rate is high (3–10% depending on the definition of a significant malformation) and highly variable between different regions, age groups and social classes. Large-scale long-term studies are required, and the results are often inconclusive.

▼ Studies using embryonic stem cells in assessing developmental toxicity are showing some promise. *In vitro* methods, based on the culture of cells, organs or whole embryos, have, however, not so far been developed to a level where they satisfactorily predict teratogenesis *in vivo*, and most regulatory authorities require teratogenicity testing in a rodent and a non-rodent species (e.g. rabbit). Pregnant females are dosed at various levels during the critical period of organogenesis, and the fetuses are examined for structural abnormalities. However, poor cross-species correlation means that tests of this kind are not reliably predictive in humans, and it is usually recommended that new drugs are not used in pregnancy unless it is essential.

SOME DEFINITE AND PROBABLE HUMAN TERATOGENS

Although many drugs have been found to be teratogenic in varying degrees in experimental animals, relatively few are known to be teratogenic in humans (see Table 57.2). Some of the more important are discussed below.

Thalidomide

Thalidomide is almost unique in producing, at therapeutic dosage, virtually 100% malformed infants when taken in the first 3–6 weeks of gestation. It was introduced in 1957 as a hypnotic and sedative with the special feature that it was much less hazardous in overdosage than barbiturates, and it was even recommended specifically for use in pregnancy (with the advertising slogan 'the safe hypnotic'). It had been subjected to toxicity testing only in mice, which are resistant to thalidomide teratogenicity. Thalidomide was marketed energetically and successfully, and the first suspicion of its teratogenicity arose early in 1961 with reports of a sudden increase in the incidence of phocomelia ('seal limbs', an absence of development of the long bones of the arms and legs) that had hitherto been virtually unknown. At this time, a million tablets were being sold daily in West Germany. Reports of phocomelia came simultaneously from Hamburg and Sydney, and the connection with thalidomide was made.[3] The drug was withdrawn late in 1961, by which time an estimated 10 000 malformed babies had been born (Fig. 57.3 illustrates the use of data linkage in detecting delayed ADRs). Despite intensive study, its mechanism remains poorly understood, although epidemiological

Fig. 57.3 Incidence of major fetal abnormalities in Western Europe following the introduction and withdrawal of thalidomide linked to sales data for thalidomide.

Table 57.4 Thalidomide teratogenesis

Day of gestation	Type of deformity
21–22	Malformation of ears Cranial nerve defects
24–27	Phocomelia of arms
28–29	Phocomelia of arms and legs
30–36	Malformation of hands Anorectal stenosis

investigation showed very clearly the correlation between the time of exposure and the type of malfunction produced (Table 57.4).

Cytotoxic drugs

Many alkylating agents (e.g. **chlorambucil** and **cyclophosphamide**) and antimetabolites (e.g. **azathioprine** and **mercaptopurine**) cause malformations when used in early pregnancy but more often lead to abortion (see Ch. 56). Folate antagonists (e.g. **methotrexate**) produce a much higher incidence of major malformations, evident in both live-born and stillborn fetuses.

Retinoids

Etretinate, a retinoid (i.e. vitamin A derivative) with marked effects on epidermal differentiation, is a known teratogen and causes a high proportion of serious abnormalities (notably skeletal deformities) in exposed fetuses. Dermatologists use retinoids to treat skin diseases, including several, such as acne and psoriasis, that are common in young women. Etretinate accumulates in subcutaneous fat and is eliminated extremely slowly, detectable amounts persisting for many months after chronic dosing is discontinued. Because of this, women should avoid pregnancy for at least 2 years after treatment. **Acitretin** is an active metabolite of etretinate. It is equally teratogenic, but tissue accumulation is less pronounced and elimination may be more rapid.

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[3]A severe peripheral neuropathy, leading to irreversible paralysis and sensory loss, was reported within a year of the drug's introduction and subsequently confirmed in many reports. The drug company responsible was less than punctilious in acting on these reports (see Sjöström & Nilsson, 1972), which were soon eclipsed by the discovery of teratogenic effects, but the neurotoxic effect was severe enough in its own right to have necessitated restriction of the drug from general use. Today, use of thalidomide has had a resurgence related to several highly specialised applications. It is prescribed by specialists (in dermatology, oncology and in HIV infection, among others) under tightly controlled and restricted conditions.
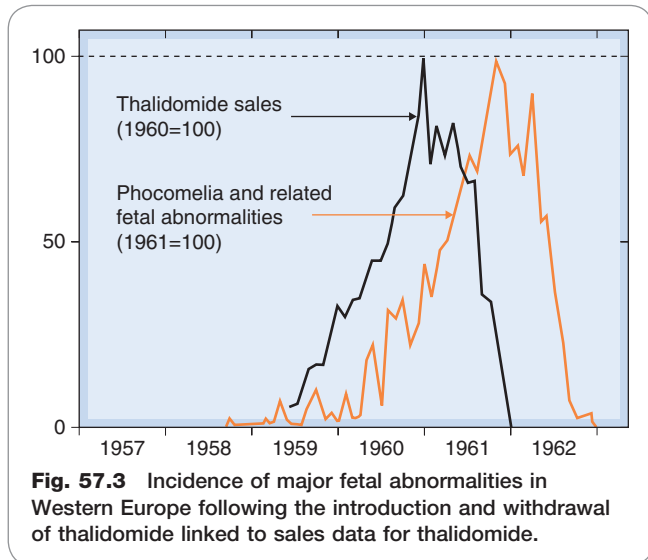
Heavy metals

Lead, *cadmium* and *mercury* all cause fetal malformation in humans. The main evidence comes from *Minamata disease*, named after the locality in Japan where an epidemic occurred when the local population ate fish contaminated with methylmercury that had been used as an agricultural fungicide. This impaired brain development in exposed fetuses, resulting in cerebral palsy and mental retardation, often with microcephaly. Mercury, like other heavy metals, inactivates many enzymes by forming covalent bonds with sulfhydryl and other groups, and this is believed to be responsible for these developmental abnormalities.

Antiepileptic drugs (see Ch. 45)

Congenital malformations are increased two- to three-fold in babies of epileptic mothers, especially of mothers treated with two or more antiepileptic drugs during the first trimester, and in association with above-therapeutic plasma concentrations. Many antiepileptic drugs have been implicated, including **phenytoin** (particularly cleft lip/palate), **valproate** (neural tube defects) and **carbamazepine** (spina bifida and hypospadias, a malformation of the male urethra), as well as newer agents including **lamotrigine** and **topiramate** (Ch. 45). The relative risks attributable to different antiepileptic drugs are not well defined, but there is evidence that valproate is particularly harmful.

Warfarin

Administration of **warfarin** (Ch. 24) in the first trimester is associated with nasal hypoplasia and various central nervous system abnormalities, affecting roughly 25% of exposed babies. In the last trimester, it must not be used because of the risk of intracranial haemorrhage in the baby during delivery.

> **Teratogenesis and drug-induced fetal damage**
>
> - Teratogenesis means production of gross structural malformations of the fetus (e.g. the absence of limbs after **thalidomide**). Less comprehensive damage can be produced by several drugs (see Table 57.2). Less than 1% of congenital fetal defects are attributed to drugs given to the mother.
> - Gross malformations are produced only if teratogens act during organogenesis. This occurs during the first 3 months of pregnancy but after blastocyst formation. Drug-induced fetal damage is rare during blastocyst formation (exception: fetal alcohol syndrome) and after the first 3 months (exception: ACE inhibitors and sartans).
> - The mechanisms of action of teratogens are not clearly understood, although DNA damage is a factor.

IMMUNOLOGICAL REACTIONS TO DRUGS

Biological agents (Ch. 59) may provoke an immune response; anti-drug antibodies to insulin are common in diabetic patients, though they seldom cause problems (Ch. 31), but antidrug antibodies to erythropoietin and to thrombopoietin can have serious consequences for patients treated with these agents. (see Ch. 25). Measurement of antidrug antibodies is now routine

during development of biological products. Seemingly trivial differences in manufacturing process (e.g. between different batches, or when a new manufacturer makes a copy of a biological product after it is no longer protected by patent – so-called 'biosimilar' products) can result in marked changes in immunogenicity.

Allergic reactions of various kinds are a common form of adverse drug reaction. Low-molecular-weight drugs are not immunogenic in themselves. A drug or its metabolites can, however, act as a *hapten* by interacting with protein to form a stable immunogenic conjugate (Ch. 6). The immunological basis of some allergic drug reactions has been well worked out, but often it is inferred from the clinical characteristics of the reaction, and direct evidence of an immunological mechanism is lacking. The existence of an allergic reaction is suggested by its delayed onset, or occurrence only after repeated exposure to the drug. Allergic reactions are generally unrelated to the main action of the drug, and conform to syndromes associated with types I, II, III and IV of the Gell and Coombs classification (see below and Ch. 6).

The overall incidence of allergic drug reactions is variously reported as being between 2% and 25%. Most are minor skin eruptions. Serious reactions (e.g. anaphylaxis, haemolysis and bone marrow depression) are rare. Penicillins, which are the commonest cause of drug-induced anaphylaxis, produce this response in an estimated 1 in 50 000 patients exposed. Rashes can be severe, and fatalities occur with Stevens–Johnson syndrome (provoked, for example, by sulfonamides), and toxic epidermal necrolysis (TEN, which can be caused for example by **allopurinol**). The association between **carbamazepine**-induced TEN and the gene for a particular human leukocyte antigen (HLA) allele *HLAB*1502* in people of Asian ancestry is mentioned in Chapter 11. Susceptibility to severe rashes in response to **abacavir** is closely linked to the variant *HLAB*5701* and this forms the basis of a clinically useful genomic test (Ch. 11).

IMMUNOLOGICAL MECHANISMS

The formation of an immunogenic conjugate between a small molecule and an endogenous protein requires covalent bonding. In most cases, reactive metabolites, rather than the drug itself, are responsible. Such reactive metabolites can be produced during drug oxidation or by photoactivation in the skin. They may also be produced by the action of toxic oxygen metabolites generated by activated leukocytes. Rarely (e.g. in drug-induced lupus erythematosus), the reactive moiety interacts to form an immunogen with nuclear components (DNA, histone) rather than proteins. Conjugation with a macromolecule is usually essential, although penicillin is an exception because it can form sufficiently large polymers in solution to elicit an anaphylactic reaction in a sensitised individual even without conjugation to protein, although penicillin–protein conjugates can also act as the immunogen.

CLINICAL TYPES OF ALLERGIC RESPONSE TO DRUGS

Hypersensitivity reactions of types I, II and III (Ch. 6) are antibody-mediated reactions while type IV is cell-mediated. Unwanted reactions to drugs involve both antibody- and cell-mediated reactions. The more important

clinical manifestations of hypersensitivity include anaphylactic shock, haematological reactions, allergic liver damage and other hypersensitivity reactions.

ANAPHYLACTIC SHOCK

Anaphylactic shock – see also Chapters 6 and 28 – is a type I hypersensitivity response. It is a sudden and life-threatening reaction that results from the release of histamine, leukotrienes and other mediators. The main features include urticarial rash, swelling of soft tissues, bronchoconstriction and hypotension.

Penicillins account for about 75% of anaphylactic deaths, reflecting the frequency with which they are used in clinical practice. Other drugs that can cause anaphylaxis include enzymes, such as **asparaginase** (Ch. 56); therapeutic monoclonal antibodies (Ch. 59); hormones, for example **corticotropin** (Ch. 33); **heparin** (Ch. 24); dextrans; radiological contrast agents; vaccines; and other serological products. Anaphylaxis with local anaesthetics (Ch. 43), the antiseptic chlorhexidine and with many other drugs (sometimes as a consequence of contaminants such as latex used to seal reusable vials or of excipients and colouring agents rather than the drug itself) can occur. Treatment of anaphylaxis is mentioned in Chapter 28.

It is sometimes feasible to carry out a skin test for the presence of hypersensitivity, which involves injecting a minute dose intradermally. A patient who reports that she or he is allergic to a drug such as penicillin may actually be allergic to fungal contaminants, which were common in early preparations, rather than to penicillin itself. The use of penicilloylpolylysine as a skin test reagent for penicillin allergy is an improvement over the use of penicillin itself, because it bypasses the need for conjugation of the test substance, thereby reducing the likelihood of a false negative. Other specialised tests are available to detect the presence of specific immunoglobulin E in the plasma, or to measure histamine release from the patient's basophils, but these are not used routinely.

HAEMATOLOGICAL REACTIONS

Drug-induced haematological reactions can be produced by type II, III or IV hypersensitivity. Type II reactions can affect any or all of the formed elements of the blood, which may be destroyed by effects either on the circulating blood cells themselves or on their progenitors in the bone marrow. They involve antibody binding to a drug–macromolecule complex on the cell surface membrane. The antigen–antibody reaction activates complement, leading to lysis, or provokes attack by killer lymphocytes or phagocytic leukocytes (Ch. 6). *Haemolytic anaemia* has been most commonly reported with sulfonamides and related drugs (Ch. 51) and with an antihypertensive drug, **methyldopa** (Ch. 14), which is still widely used to treat hypertension during pregnancy. With methyldopa, significant haemolysis occurs in less than 1% of patients, but the appearance of antibodies directed against the surface of red cells is detectable in 15% by the Coombs test. The antibodies are directed against Rh antigens, but it is not known how methyldopa produces this effect.

Drug-induced *agranulocytosis* (complete absence of circulating neutrophils) is usually delayed 2–12 weeks after beginning drug treatment but may then be sudden in onset. It often presents with mouth ulcers, a severe sore throat or other infection. Serum from the patient lyses leukocytes from other individuals, and circulating anti-leukocyte antibodies can usually be detected immunologically. Drugs associated with agranulocytosis include NSAIDs, especially **phenylbutazone** (Ch. 26), **carbimazole** (Ch. 34) and **clozapine** (Ch. 46) (increased genetic susceptibility associated with *HLA-DQB1*0201* is mentioned in Ch. 11) and **sulfonamides** and related drugs (e.g. *thiazides* and *sulfonylureas*). Agranulocytosis is rare but life-threatening. Recovery when the offending drug is stopped is often slow or absent. Antibody-mediated leukocyte destruction must be distinguished from the direct effect of cytotoxic drugs (see Ch. 55), which cause granulocytopenia that is rapid in onset, predictably related to dose and reversible.

Thrombocytopenia (reduction in platelet numbers) can be caused by type II reactions to **quinine** (Ch. 54), **heparin** (Ch. 24) and thiazide diuretics (Ch. 29).

Some drugs (notably **chloramphenicol**) can suppress all three haemopoietic cell lineages, giving rise to *aplastic anaemia* (anaemia with associated agranulocytosis and thrombocytopenia).

The distinction between type III and type IV hypersensitivity reactions in the causation of haematological reactions is not clear-cut, and either or both mechanisms can be involved.

ALLERGIC LIVER DAMAGE

Most drug-induced liver damage results from the direct toxic effects of drugs or their metabolites, as described above. However, hypersensitivity reactions are sometimes involved, a particular example being **halothane**-induced hepatic necrosis (see Ch. 41). *Trifluoracetylchloride*, a reactive metabolite of halothane, couples to a macromolecule to form an immunogen. Most patients with halothane-induced liver damage have antibodies that react with halothane–carrier conjugates. Halothane–protein antigens can be expressed on the surface of hepatocytes. Destruction of the cells occurs by type II hypersensitivity reactions involving killer T cells, and type III reactions can also contribute.

OTHER HYPERSENSITIVITY REACTIONS

The clinical manifestations of type IV hypersensitivity reactions are diverse, ranging from minor skin rashes to generalised autoimmune disease. Fever may accompany these reactions. Rashes can be antibody mediated but are usually cell mediated. They range from mild eruptions to fatal exfoliation. Stevens–Johnson syndrome is a very severe generalised rash that extends into the alimentary tract and carries an appreciable mortality. In some cases, the lesions are photosensitive, probably because ultraviolet light converts the drug to reactive products.

▼ Some drugs (notably **hydralazine** and **procainamide**) can produce an autoimmune syndrome resembling systemic lupus erythematosus. This is a multisystem disorder in which there is immunological damage to many organs and tissues (including joints, skin, lung, central nervous system and kidney) caused particularly, but not exclusively, by type III hypersensitivity reactions. The prodigious array of antibodies directed against 'self' components has been termed an 'autoimmune thunderstorm'. The antibodies react with determinants shared by many molecules, for example the phosphodiester backbone of DNA, RNA and phospholipids. In drug-induced systemic lupus erythematosus, the immunogen may result from the reactive drug moiety interacting with nuclear material, and joint and pulmonary damage is common. The condition usually resolves when treatment with the offending drug is stopped.

Allergic reactions to drugs

- Drugs or their reactive metabolites can bind covalently to proteins to form immunogens. **Penicillin** (which can also form immunogenic polymers) is an important example.
- Drug-induced allergic (hypersensitivity) reactions may be antibody-mediated (types I, II, III) or cell-mediated (type IV). Important clinical manifestations include the following:
  – anaphylactic shock (type I): many drugs can cause this, and most deaths are caused by **penicillin**
  – haematological reactions (type II, III or IV): including haemolytic anaemia (e.g. **methyldopa**),

agranulocytosis (e.g. **carbimazole**), thrombocytopenia (e.g. **quinine**) and aplastic anaemia (e.g. **chloramphenicol**)
  – hepatitis (types II, III): for example, **halothane**, **phenytoin**
  – rashes (type I, IV): are usually mild but can be life-threatening (e.g. Stevens–Johnson syndrome)
  – drug-induced systemic lupus erythematosus (mainly type II): antibodies to nuclear material are formed (e.g. **hydralazine**).

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Lifestyle drugs and drugs in sport **58**

OVERVIEW

The term *lifestyle* is applied to drugs that are used for non-medical purposes. This is a diverse group that includes drugs of abuse, drugs used to enhance athletic or other performance, as well as those taken for cosmetic purposes or for purely social reasons. Many lifestyle drugs have dual uses and are also employed as conventional therapeutics and their pharmacological properties are described elsewhere in this book. In this chapter we present an overall summary of lifestyle drugs and discuss some of the social and medico-legal problems associated with their growing use.

Drugs that are used to enhance sporting performance, while being officially prohibited, represent a special category of lifestyle drugs. Once again, many types of substances are used for this purpose, including established medicines. Below, we discuss specific issues relating to their use in competitive sports.

WHAT ARE LIFESTYLE DRUGS?

This is a question that is sometimes difficult to answer. Here we define them as drugs or medicines that are taken by choice to give pleasure (e.g. cannabis, alcohol, cocaine), to improve performance (e.g. drugs in sport, cognition-enhancing drugs) or to improve appearance (e.g. **botox**, slimming aids for the non-obese), in other words to satisfy an aspiration or a non-health-related goal rather than to treat a clinical condition. Put simply, they are drugs taken by choice by people who are not ill. Examples include the use of the antihypertensive **minoxidil** for treating baldness. Oral contraceptives, which clearly lie in the domain of mainstream medicine, could also be considered lifestyle drugs. Also included in the lifestyle category are food supplements and other related preparations that are consumed because of some claimed benefit – even though there is often no good evidence that they are effective.

CLASSIFICATION OF LIFESTYLE DRUGS

The lifestyle category covers lifestyle *uses* of a wide variety of drugs and medicines and cuts across the pharmacological classification used throughout this book, so summarising it is difficult. The scheme in Table 58.1 is based largely on the work of Gilbert et al. (2000) and Young (2003). It embraces drugs that have been used for lifestyle choices based on historical precedent, such as oral contraceptives, as well as agents used to manage potentially debilitating lifestyle illnesses such as addiction to smoking (e.g. **bupropion**). It also includes drugs such as caffeine and alcohol that are consumed on a mass scale around the world, and drugs of abuse such as cocaine as well as nutritional supplements. Particularly topical is the

controversial use of 'neuro-enhancers', such as **modafinil** and **methylphenidate** (Ch. 48), which are claimed to improve academic performance (see Sahakian & Morein-Zamir, 2007; Eickenhorst et al., 2012, for example), although much evidence is anecdotal.[1]

Over time, drugs can switch between 'lifestyle' and 'clinical' uses. For example, **cocaine** was used as a lifestyle drug by South American Indians. Early explorers commented that it 'satisfies the hungry, gives new strength to the weary and exhausted and makes the unhappy forget their sorrows'. Subsequently assimilated into European medicine as a local anaesthetic (Ch. 43), it is now largely returned to lifestyle drug status and, regrettably, is the basis of an illegal multimillion dollar international drugs industry. **Cannabis** is another good example of a drug that has been considered (in the West at least) as a purely recreational drug but which is now (as a plant extract containing **tetrahydrocannabinol** and **cannabidiol**) licensed for various clinical uses (see Chs 19, 42 and 49). There are many other examples (Flower, 2004).

Many widely used lifestyle 'drugs' or 'sports supplements' consist of natural products (e.g. *Ginkgo* extracts, melatonin, St John's wort, *Cinchona* extracts), whose manufacture and sale has not generally been controlled by regulatory bodies.[2] Their composition is therefore highly variable, and their efficacy and safety generally untested. Many contain active substances that, like synthetic drugs, can produce adverse as well as beneficial effects.

DRUGS IN SPORT

The American cyclist Lance Armstrong seemed to be an inspirational hero. Having overcome testicular cancer he went on to win the Tour de France on no less than seven occasions and the charity he founded raised millions of dollars for cancer relief. Persistent accusations of drug abuse surrounded the athlete but were strenuously denied, until January 2013 when Armstrong admitted, on a television chat show, that he had been using a cocktail of drugs to enhance his performance over the course of many years.[3] It prompted one commentator (Sparling, 2013) to despair of the 'charade of drug-free sport'.

The use of drugs to enhance sporting performance is evidently widespread, although officially prohibited.

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[1]Drugs intended to give a competitive advantage in sport are, of course, considered unfair, banned and very actively policed. Will there come a time when taking drugs to improve examination performance will become illegal, with similar surveillance methods and sanctions? See Bostrom & Sandberg (2009) for a discussion of this ethical minefield.
[2]Things are changing. In the UK, the Medicines and Healthcare Products Regulatory Agency now has a Herbal Medicines Advisory Committee.
[3]Apparently including steroids, growth hormone and erythropoietin. He was later stripped of all his sporting honours.

Table 58.1   Lifestyle drugs and medicines, excluding drugs in sport

Category	Example(s)	Primary clinical use	'Lifestyle' use	Chapter
Medicines approved for specific indications but which also have other 'lifestyle' purposes	Sildenafil	Erectile dysfunction	Erectile enhancement	35
	Oral contraceptives	Preventing conception	Preventing conception	35
	Orlistat	Obesity	Weight loss	32
	Sibutramine	Anorectic agent (withdrawn in Europe)	Weight loss	32
Medicines approved for specific indications which can also be used to satisfy 'lifestyle choices' or to treat 'lifestyle diseases'	Minoxidil	Hypertension	Regrowth of hair	22
	Methylphenidate	Attention deficit/hyperactivity disorder (ADHD)	Improving academic performance	48
	Modafinil	Treatment of ADHD	Cognitive enhancement	48
	Opiates	Analgesia	'Recreational' usage	42, 49
Drugs that have only slight, or no, current clinical use but which fall into the lifestyle category	Alcohol	None as such	Widespread component of drinks	49
	Botulinum toxin	Relief of muscle spasm	Cosmetic alteration	13
	Caffeine	Migraine treatment	Widespread component of drinks	48
	Cannabis	Managing chronic pain, nausea and possibly muscle spasm	'Recreational' usage	19, 49
Drugs (generally illegal) that have no clinical utility but which are used to satisfy lifestyle requirements	Methylenedioxymethamphetamine (MDMA, 'ecstasy')	None	'Recreational' usage	48
	Tobacco (nicotine)	Nicotine preparations for tobacco addiction	'Recreational' usage	49
	Cocaine (some formulations)	Local anaesthesia (largely obsolete)	'Recreational' usage	42

In addition, there are countless herbal preparations and other natural products, largely unregulated, which are marketed as health-promoting, life-enhancing and beneficial for many disorders, despite lack of evidence of therapeutic efficacy. Many are claimed to 'boost the immune system'. Examples include numerous vitamin preparations, fish oils, melatonin, ginseng, *Echinacea*, *Ginkgo* and much besides.

From *Flower 2004*, after *Gilbert et al., 2000* and *Young, 2003*.

Lifestyle drugs

- Comprise a group of drugs and medicines taken mainly for non-medical reasons. Should more accurately be called 'lifestyle uses'.
- Include prescription drugs such as **sildenafil** and **methylphenidate**, substances such as **alcohol** and **caffeine**, drugs of abuse and various nutritional preparations.
- Are linked to the concepts of 'self-diagnosis' and 'non-disease'.
- Are a growing sector of the pharmaceutical market.
- Are often brought to the consumer's attention through the Internet or direct marketing of drugs.

The World Anti-Doping Agency (www.wada-ama.org), which was established partly in response to some high-profile doping cases and drug-induced deaths among athletes, publishes an annually updated list of prohibited substances that may not be used by sportsmen or sportswomen either in, or out of, competition. Drug testing is based mainly on analysis of blood or urine samples according to strictly defined protocols. The chemical analyses, which rely mainly on gas chromatography/mass spectrometry or immunoassay techniques, must be carried out by approved laboratories.

Table 58.2 summarises the main classes of drugs that are prohibited for use in sports. Athletes are easily persuaded of the potential of a wide variety of drugs to increase their chances of winning, but it should be emphasised that in very few cases have controlled trials shown that the drugs actually improve sporting performance among trained athletes, and indeed many such trials have proved negative. However, marginal improvements in performance (often 1% or less), which are difficult to measure experimentally, may make the difference between winning and losing, and the competitive instincts of athletes and their trainers generally carry more weight than scientific evidence.

A brief account of some of the more important drugs in common use follows. For a broader and more complete coverage, see British Medical Association (2002) and

Table 58.2   Drugs used in sport

Drug class	Example(s)	Effects	Detection	Notes
Anabolic agents	Androgenic steroids (testosterone, nandrolone and many others; Ch. 35)	Increased muscle development, aggression and competitiveness Serious long-term side effects	Urine or blood samples	Many are endogenous hormones, so results significantly above normal range are required
	Clenbuterol (Ch. 14)	Combined anabolic and agonist action on β_2 adrenoceptors may increase muscle strength		Human chorionic gonadotrophin is sometimes used to increase androgen secretion
Hormones and related substances	Erythropoietin (Ch. 25)	Increased erythrocyte formation and oxygen transport. Increased blood viscosity causes hypertension and risk of strokes and coronary attacks Used mainly for endurance sports[a]	Plasma half-life is short, so detection is difficult	Use of other plasma markers indicating erythropoietin administration may be possible
	Human growth hormone (Ch. 33)	Increased lean body mass and reduced fat May accelerate recovery from tissue injury. Causes cardiac hypertrophy, acromegaly, liver damage and increased cancer risk	Blood testing	Distinguishing endogenous (highly variable) from exogenous human growth hormone can be difficult
	Insulin (Ch. 31)	Sometimes used (with glucose so as to avoid hypoglycaemia) to promote glucose uptake and energy production in muscle Probably ineffective in improving performance	Plasma samples	–
β_2-Adrenoceptor agonists	Salbutamol and others (Ch. 14)	Used by runners, cyclists, swimmers, etc. to increase oxygen uptake (by bronchodilatation) and increased cardiac function. Controlled studies show no improvement in performance	Urine samples	–
β-Adrenoceptor antagonists	Propranolol, etc. (Ch. 14)	Used to reduce tremor and anxiety in 'precision' sports (e.g. shooting, gymnastics, diving)	Urine samples	Not banned in most sports, where they actually impair performance
CNS 'stimulants'	Ephedrine and derivatives; amphetamines, cocaine, caffeine (Ch. 48)	Many trials show slight increase in muscle strength and performance in non-endurance events (sprint, swimming, field events, etc.)	Urine samples	The most widely used group, along with anabolic steroids
Diuretics	Thiazides, furosemide (Ch. 29)	Used mainly to achieve rapid weight loss before 'weighing in'. Also used to 'mask' the presence of other agents in urine by dilution	Urine samples	–
Narcotic analgesics	Codeine, morphine, etc. (Ch. 42)	Used to mask injury-associated pain	Urine samples	–

[a]'Blood doping' (removal of 1–2 l of blood well ahead of the competition, followed by re-transfusion immediately prior to the event) has a similar effect and is even more difficult to detect.

Mottram (2005). Gould (2013) has reviewed the potential use of gene therapy in promoting athletic performance. Another hurdle for the regulators!

ANABOLIC STEROIDS

Anabolic steroids (Ch. 35) include a large group of compounds with testosterone-like effects, including about 50 named compounds on the prohibited list. New chemical derivatives ('designer steroids'), such as **tetrahydrogestrinone** (THG), are regularly developed and offered illicitly to athletes, which represents a continuing problem to the authorities charged with detecting and identifying them. A further problem is that some of these drugs are endogenous compounds or their metabolites and their concentration can vary dramatically for physiological reasons. This makes it difficult to prove that the substance had been administered illegally. Isotope ratio techniques,

based on the fact that endogenous and exogenous steroids have slightly different $^{12}C:^{13}C$ ratios, may enable the two to be distinguished analytically. Since anabolic steroids produce long-term effects and are normally used throughout training, rather than during the event itself, out-of-competition testing is essential.

When given in combination with training and high protein intake, anabolic steroids undoubtedly increase muscle mass and body weight, but there is little evidence that they increase muscle strength over and above the effect training could achieve alone, or that they improve sporting performance. On the other hand, they have serious long-term effects, including male infertility, female masculinisation, liver and kidney tumours, hypertension and increased cardiovascular risk, and (in adolescents) premature skeletal maturation causing irreversible cessation of growth. Anabolic steroids produce a feeling of physical well-being, increased competitiveness and aggressiveness, sometimes progressing to actual psychosis. Depression is common when the drugs are stopped, sometimes leading to long-term psychiatric problems.

Clenbuterol, is a β-adrenoceptor agonist (see Ch. 14). Through an unknown mechanism of action, it produces anabolic effects similar to those of androgenic steroids, with apparently fewer adverse effects. It can be detected in urine and its use in sport is banned.

HUMAN GROWTH HORMONE

The use of **human growth hormone** (hGH; see Ch. 33) by athletes followed the availability of the recombinant form of hGH, used to treat endocrine disorders. It is given by injection and its effects appear to be similar to those of anabolic steroids. hGH is also reported to produce a similar feeling of well-being, although without the accompanying aggression and changes in sexual development and behaviour. It increases lean body mass and reduces body fat, but its effects on muscle strength and athletic performance are unclear. It is claimed to increase the rate of recovery from tissue injury, allowing more intensive training routines. The main adverse effect of hGH is the development of acromegaly, causing overgrowth of the jaw and thickening of the fingers (Ch. 33), but it may also lead to cardiac hypertrophy and cardiomyopathy, and possibly also an increased cancer risk.

Detection of hGH administration is difficult because physiological secretion is pulsatile, so normal plasma concentrations vary widely. The plasma half-life is short (20–30 min), and only trace amounts are excreted in urine. However, secreted hGH consists of three isoforms varying in molecular weight, whereas recombinant hGH contains only one, so measuring the relative amounts of the isoforms can be used to detect the exogenous material. Growth hormone acts partly by releasing insulin-like growth factor from the liver, and this hormone itself is coming into use by athletes.

Another hormone, **erythropoietin**, which increases erythrocyte production (see Ch. 25), is given by injection for days or weeks to increase the erythrocyte count and hence boost the O_2-carrying capacity of blood. The development of recombinant erythropoietin has made it widely available, and detection of its use is difficult. It carries a risk of hypertension, neurologic disease and thrombosis.

STIMULANT DRUGS

The main drugs of this type used by athletes and officially prohibited are: **ephedrine** and **methylephedrine**; various amphetamines and similar drugs, such as **fenfluramine** and methylphenidate;[4] cocaine; and a variety of other CNS stimulants such as **nikethamide**, **amiphenazole** (no longer used clinically) and **strychnine** (see Ch. 48). **Caffeine** is also used: some commercially available 'energy drinks' contain taurine as well as caffeine. However, taurine is an agonist at glycine and extrasynaptic $GABA_A$ receptors (see Ch. 39). Its effects on the brain are therefore likely to be inhibitory rather than stimulatory. In this regard, taurine may be responsible for the post-energy-drink low that is experienced once the stimulatory effect of caffeine has worn off.

In contrast to steroids, some trials have shown stimulant drugs to improve performance in events such as sprinting and weightlifting, and under experimental conditions they increase muscle strength and reduce muscle fatigue significantly. The psychological effect of stimulants is probably more relevant than their physiological effects. Surprisingly, caffeine appears to be more consistently effective in improving muscle performance than other more powerful stimulants.

Several deaths have occurred among athletes taking amphetamines and ephedrine-like drugs in endurance events. The main causes are coronary insufficiency, associated with hypertension; hyperthermia, associated with cutaneous vasoconstriction; and dehydration.

From a pharmacological perspective, it is fair to say that the use of drugs to enhance sporting performance carries many risks and is of doubtful efficacy. Its growing prevalence reflects many of the same pressures as those driving the introduction of lifestyle drugs, namely the desire to enhance the performance of humans who are not impaired by disease, coupled with disregard for scientific evidence relating to efficacy and risk.

CONCLUSION

The lifestyle drug phenomenon is one aspect of a broader debate about what actually constitutes 'disease' and how far medical science should go to satisfy the needs and aspirations of otherwise healthy individuals or to alleviate human distress and dysfunction in the absence of pathology. Discussion of these issues is beyond the scope of this book but can be found in articles cited at the end of this chapter (see Flower 2004 and 2012).

There are several reasons why these drugs – no matter how we choose to define them – are of increasing concern. The increasing availability of drugs from 'e-pharmacies', coupled with the direct advertising by the pharmaceutical industry to the public that occurs in some countries, will ensure that demand is kept buoyant. Most sales are in the developed world and the pharmaceutical industry will undoubtedly develop more lifestyle agents to cater for this lucrative market. The lobbying power of patients advocating particular drugs, regardless of the potential costs or proven utility, causes major problems for drug regulators and those who set healthcare priorities for state-funded systems of social medicine.

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[4]Also used to improve academic performance!

Drugs in sport

- Many drugs of different types are commonly used by sportsmen and sportswomen with the aim of improving performance in competition.
- The main types used are:
  - anabolic agents, mainly androgenic steroids and **clenbuterol**
  - hormones, particularly **erythropoietin** and **human growth hormone**
  - stimulants, mainly **amphetamine** and **ephedrine** derivatives and caffeine
  - β-adrenoceptor antagonists, to reduce anxiety and tremor in 'precision' sports.

- The use of drugs in sport is officially prohibited – in most cases, in or out of competition.
- Detection depends mainly on analysis of the drug or its metabolites in urine or blood samples. Detection of abuse is difficult in the case of endogenous hormones such as **erythropoietin**, **growth hormone** and **testosterone**.
- Controlled trials have mostly shown that drugs produce little improvement in sporting performance. Anabolic agents increase body weight and muscle volume without clearly increasing strength. The effect of stimulants is often psychological rather than physiological.

The use of drugs that improve short-term memory to treat patients with dementia (Ch. 40) is generally seen as desirable (even though current drugs are only marginally effective). Extending the use of existing and future drugs to give healthy children and students a competitive advantage in tests is much more controversial. Further off is the prospect of drugs that retard senescence and prolong life – another social and ethical minefield in an overpopulated world.

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59 Biopharmaceuticals and gene therapy

OVERVIEW

In this chapter, we review the impact of two therapeutic concepts based on our growing understanding and skill in manipulating genes. *Biopharmaceuticals* is an umbrella term applied to the use of 'engineered' proteins and antibodies or nucleic acids in medicine, while *gene therapy* refers specifically to attempts to use genes to reprogram cells to prevent, alleviate or cure disease. Engineered proteins, after some 30 years of (often frustrating) research and development, are well established in the clinic, while nucleic acid drugs and gene therapy are still in development.[1] In addition to introducing the central concepts in this chapter, we discuss the considerable problems associated with developing biopharmaceutical therapies, consider safety issues and review the progress made to date.

INTRODUCTION

The 'molecular biology revolution', which had its roots in the discovery of the structure of DNA in the 1950s, and the advances in cell biology that followed in its train, offered the prospect of manipulating the genetic material in ways that are useful in practical therapeutics. The seductive notion that a gene of interest can be expressed *in vitro* to generate useful proteins that could not be prepared synthetically or, more daringly, that a gene could be directly introduced *in vivo* and persuaded to synthesise some crucial cellular component, has driven this field at breakneck speed.

Biopharmaceuticals (often called *biologics*) are by now a well-recognised part of therapy, and we have already encountered them elsewhere in this book (see Tables 59.1 and 59.2). Widespread adoption of these drugs still faces many problems, not the least of which is the cost of manufacture, but the technology is now established and maturing fast. In a review of the area published in 2013, Wirth noted that some 211 biopharmaceuticals had been licensed around the world by 2011, earning some $113 billion revenue.[2] The veterinary use of these drugs is also increasing.

Gene therapy is the more considerable challenge. However, the idea commands such appeal that vast resources (both public and private) have been committed to its development. There are several reasons why it is so attractive. First, it is a (deceptively) simple approach to a radical cure of single-gene diseases such as *cystic fibrosis* and the *haemoglobinopathies*, which are collectively responsible for much misery throughout the world. Second, many other more common conditions, including malignant, neurodegenerative and infectious diseases, have a large genetic component. Conventional treatment of such disorders is (as readers of this book will have appreciated by now) far from ideal, so the promise of a completely new approach has enormous attraction. Finally, an ability to control gene expression (e.g. by antisense or siRNA oligonucleotides) could be used to treat many diseases that are not genetic in origin.

The gurus are emphatic that 'the conceptual part of the gene therapy revolution has indeed occurred …' – so where are the therapies? The devil, of course, is in the detail: in this case, the details of:

- *pharmacokinetics*: delivery of the gene to the interior of appropriate target cells (especially in the central nervous system [CNS])
- *pharmacodynamics*: the controlled expression of the gene in question
- *safety*
- *clinical efficacy and long-term practicability*.

The first and most fundamental hurdle is the delivery problem; here, techniques borrowed from viruses, which are masters of the sort of molecular hijacking that is required to introduce functional genes into mammalian cells, have been used.

There is a broad consensus that the *Weismann barrier*[3] should not be breached and so a moratorium has been agreed on therapies intended to alter the DNA of germ cells (which could influence future generations) and gene therapy trials have focused on somatic cells.

BIOPHARMACEUTICALS

The use of proteins as therapeutic agents is not a novel idea; insulin, extracted from animal pancreas tissue (Ch. 31), and human growth hormone, extracted at one time from human cadaver pituitary glands (Ch. 33), were among the first therapeutic proteins to be used and, for many years, such purified extracts provided the only option for treating protein hormone deficiency disorders. However, there were problems. First, there were difficulties in extraction and often disappointing yields. Second, administration of animal hormones (e.g. pig insulin) to humans could evoke an immune response. Third, there was always a danger of the transmission of infectious agents across species, or between people. This was highlighted in the 1970s, when cases of *Creutzfeldt–Jakob disease* (see Ch. 40) occurred in patients treated with human growth hormone obtained from cadavers. This serious problem was later traced to contamination of the donor pituitary glands with infectious prions (Ch. 40). The advent of 'genetic engineering' techniques offered a new way to deal with these perennial problems.

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[1]In Western countries anyway; a gene therapy product, **Gendicine**™, for treating cancer was licensed in China in 2003.
[2]Biopharmaceuticals currently comprise about 40% of new drugs approved.

[3]Named after August Weismann (1834–1914), who formulated the concept that inheritance utilises only germ, and not somatic, cells.

Table 59.1 Some examples of 'second-generation' biopharmaceuticals

Type of change	Protein	Indication	Reason for change
Altered amino acid sequence	Insulin	Diabetes	Faster-acting hormone
	Tissue plasminogen activator analogues	Thrombolysis	Longer circulating half-life
	Interferon analogue	Antiviral	Superior antiviral action
	Factor VIII analogue	Haemophilia	Smaller molecule, better activity
	Diphtheria toxin–interleukin-2 fusion protein	T-cell lymphoma	Targets toxin to appropriate cells
	Tumour necrosis factor receptor–human immunoglobulin G Fc fusion protein	Rheumatoid disease	Prolongs half-life
Altered carbohydrate residues	Glucocerebrosidase enzyme	Gaucher's disease	Promotes phagocyte uptake
	Erythropoietin analogue	Anaemia	Prolongs half-life
Covalent attachment to polyethylene glycol	Interferon	Hepatitis C	Prolongs half-life
	Human growth hormone	Acromegaly	Prolongs half-life

Modified from Walsh 2004.

Biopharmaceuticals and gene therapy: definition and potential uses

- *Biopharmaceuticals* include proteins, antibodies (and oligonucleotides) used as drugs:
  - *first-generation* biopharmaceuticals are mainly copies of endogenous proteins or antibodies, produced by recombinant DNA technology
  - *second-generation* biopharmaceuticals have been 'engineered' to improve the performance of the protein or antibody.
- Applications:
  - therapeutic monoclonal antibodies
  - recombinant hormones.
- *Gene therapy* is the genetic modification of cells to prevent, alleviate or cure disease.
- Potential applications:
  - radical cure of monogenic diseases (e.g. cystic fibrosis, haemoglobinopathies)
  - amelioration of diseases with or without a genetic component, including many malignant, neurodegenerative and infectious diseases.

PROTEINS AND POLYPEPTIDES

The biopharmaceuticals in use today are generally classified as first- or second-generation agents. *First-generation* biopharmaceuticals are usually straightforward copies of human hormones or other proteins prepared by transfecting the human gene into a suitable *expression system* (a cell line that produces the protein in good yield), harvesting and purifying the *recombinant protein* for use as a drug. The first agent to be produced in this way was human recombinant insulin in 1982.

Second-generation biopharmaceuticals are those that have been *engineered*: that is to say, either the gene has been deliberately altered prior to transfection such that

the structure of the expressed recombinant protein is changed, or some alteration is made to the purified end product. The reasons for making these changes are generally to improve some aspect of the protein's activity profile. Human recombinant insulins designed to act faster or last longer were among the first in this class to be marketed; Table 59.1 contains other examples.

Third-generation agents would be those in which macromolecules (including nucleic acid controllers of protein synthesis as well as proteins themselves) are designed from scratch to do a particular biological function. This technology is just beginning to bear fruit: **mipomersen**, the first antisense RNA product, was licensed in 2013.

Problems in manufacture

There are several problems associated with the manufacture of any type of recombinant protein, and one of the most pressing is the choice of expression system. Many recombinant proteins are expressed in bacterial systems (*Escherichia coli*, for example), which are useful because cultures grow quickly and are generally easy to manipulate. Disadvantages include the fact that the product may contain bacterial endotoxins, which must be meticulously removed before administration to patients, and that bacterial cells do not accomplish the same type of *post-translational processing* (e.g. glycosylation) as mammalian cells. This could pose problems if the protein's action is crucially dependent on this modification. To circumvent these problems, mammalian (e.g. Chinese hamster ovary, CHO) cells are also used as expression systems, although here the problem is often one of the final yields: such cells require more careful culture, grow more slowly and produce less product, all of which contributes to the cost of the final medicine.

There are, however, a number of emergent technologies that could transform the production process. The use of plants to produce recombinant proteins has attracted considerable interest (see Melnik & Stoger, 2013). Several species have shown promise, including the tobacco plant. Human genes of interest can readily be transfected into the plant by using tobacco mosaic virus as a vector; the crop grows rapidly (yields a high *biomass*) and offers a number of other advantages. Edible plants such as

lettuce and bananas could be used to deliver some orally active proteins, such as vaccines, which could then be consumed directly without the need for prior purification. Several such proteins have already been produced in plants, and some are in clinical trial (Kwon et al., 2013).

Another technology that could dramatically increase the yield of human recombinant proteins is the use of transgenic cattle. A dairy cow can produce some 10 000 litres of milk per year, and recombinant proteins introduced into the genome, and under the control of promoters that regulate production of other milk proteins, can generate yields as high as 1 g/l (see Brink et al., 2000).

Engineered proteins

There are several ways in which proteins can be altered prior to expression. Alteration of the nucleotide sequence of the coding gene can be used to change single amino acids or, indeed, whole regions of the polypeptide chain. There are good reasons why it is an advantage to 'engineer' proteins prior to expression, including:

- modification of pharmacokinetic properties
- creation of novel *fusion* or other proteins
- reducing immunogenicity, e.g. by *humanising*.

It is frequently advantageous to modify the pharmacokinetic properties of recombinant proteins. Changes in the structure of human insulin, for example, provided diabetics with a form of the hormone that did not self-associate during storage and was thus faster-acting and easier to manage. The half-life of proteins in the blood can often be extended by *PEGylation* (see Ch. 10), the addition of polyethylene glycol to the molecule. This *post-translational engineering* approach has been applied to some human hormones, such as recombinant growth hormone, interferons and others. Prolonging half-life is not merely a convenience to patients; it also reduces the overall cost of the treatment, and economic factors are important in the adoption of this type of therapy.

Fusion proteins comprise two or more proteins engineered to be expressed as one single polypeptide chain, sometimes joined by a short linker. An example is **etanercept**, an anti-inflammatory drug used in the treatment of rheumatoid arthritis and other conditions (see Ch. 26). This consists of the ligand-binding domain taken from the tumour necrosis factor receptor, joined to the Fc domain of a human immunoglobulin G antibody. The receptor moiety sequesters tumour necrosis factor (TNF) in an inactive form, while the immunoglobulin increases its persistence in the blood. Reduction of immunogenicity through bioengineering is discussed below.

MONOCLONAL ANTIBODIES

Although antibodies are used to confer *passive immunity*, there are a number of disadvantages inherent in their production and use that limit their utility. Conventionally, antisera are produced from the blood of immunised humans or animals (e.g. to collect anti-tetanus serum). Antiserum containing high levels of specific antibodies is prepared from the plasma, which can then be used therapeutically to neutralise pathogens or other dangerous substances in the blood of the patient.

Such preparations contain *polyclonal antibodies* – that is, a *polyvalent* mixture of antibodies from all the plasma cell clones that reacted to that particular antigen. The actual composition and efficacy of these varies over time, and

obviously there is a limit to how much plasma can be collected on any one occasion. In 1975, Milstein and Köhler[4] discovered a method of producing from immunised mice an immortalised *hybridoma*, a fusion of one particular lymphocytic clone with an immortalised tumour cell. This furnished a method of producing *monoclonal antibodies* – a single species of monovalent antibody – at high abundance *in vitro*. The hybridoma cell line could be retained and expanded indefinitely while preserving the integrity of its product.

Monoclonal antibodies can be classified into first- or second-generation reagents along similar lines to other proteins discussed above. First-generation monoclonals were essentially murine monoclonals (or fragments thereof), but these suffered from several drawbacks. As mouse proteins, they provoked an immune response in 50–75% of all recipients. Other limiting factors were a short half-life in the circulation and the inability of the mouse antibodies to activate human complement.

Most of these problems have been surmounted by using either *chimeric* or *humanised* monoclonals. The two terms refer to the degree to which the monoclonals have been engineered. Figure 59.1 shows how this is done; the antibody molecule consists of a *constant* domain (Fc) and the antibody-binding domain (Fab), with *hypervariable* regions that recognise and bind to the antigen in question. The genes for chimeric monoclonals are engineered to contain the cDNA of the murine Fab domain coupled with the human Fc domain sequences. This greatly (around fivefold) extends the plasma half-life (most plasma proteins turn over quite rapidly; immunoglobulins are an exception, and it is easy to see how very long-lived antibodies provide selective advantage to the host). Incorporation of human Fc sequences also improves the functionality of the antibody in human medicine. A further development (and now the preferred approach) is to replace the entire Fc and Fab region with the human equivalent with the exception of the hypervariable regions, giving a molecule which, while essentially human in nature, contains the murine antibody-binding sites. The anticancer monoclonal **herceptin** (**trastuzumab**; see Ch. 56) is an example of such an antibody, and some others are given in Table 59.2.

THE PHARMACOLOGY OF BIOPHARMACEUTICALS

We are, by now, accustomed to the concept of using proteins and antibodies therapeutically, and many of the risks associated with (for example) anti-TNF therapy are well understood (see Ch. 26). For the most part, these medicines do not cause the range of toxic effects encountered with small molecules discussed in Chapter 57, but there are still very real dangers.

▼ In 2006, for example, a UK clinical trial of a new monoclonal antibody (TGN 1412) designed to activate T cells (see Ch. 6) and thus treat B-cell lymphocytic leukaemia went badly wrong. All six subjects became severely ill following a 'cytokine storm' and suffered lasting damage. The incident provoked wide media publicity[5] and, while the subsequent investigation blamed an 'unpredictable' biological reaction, it caused many to think hard about how such trials should be conducted in the future (see Muller & Brennan, 2009). Highly specific reagents such as monoclonals intended for human use, pose a particular problem as they may not cross-react with the corresponding proteins of other species, thus evading detection in the usual preclinical animal safety screens.

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[4]They won the 1984 Nobel Prize for Physiology or Medicine for this work.
[5]One tabloid headline read: 'We saw human guinea pigs explode' (quoted by Stobbart et al., 2007).

Table 59.2 Some examples of 'second-generation' therapeutic monoclonal antibodies

Antibody	Type	Target	Use	See chapter
Infliximab	Chimeric Mab	Tumour necrosis factor	Crohn's disease, rheumatoid disease	26
Adalimumab	Humanised Mab	Tumour necrosis factor	Rheumatoid disease	26
Etanercept	Fusion protein	Tumour necrosis factor	Rheumatoid disease	26
Trastuzumab	Humanised Mab	HER2 epidermal growth factor receptor	Breast cancer	56
Palivizumab	Humanised Mab	Respiratory syncytial virus	Respiratory infections in young children	–
Omalizumab	Humanised Mab	Immunoglobulin E	Immunoglobulin E-mediated asthma	28
Abatacept	Fusion protein	B7 epitope on antigen presenting cells	Rheumatoid disease	26

Mab, monoclonal antibody. Therapeutic monoclonal antibody names all end in '-mab', prefixed by an indication of their species nature: -umab (human), -omab (mouse), -ximab (chimera), -zumab (humanised).
Source: Walsh 2004 and the British National Formulary.
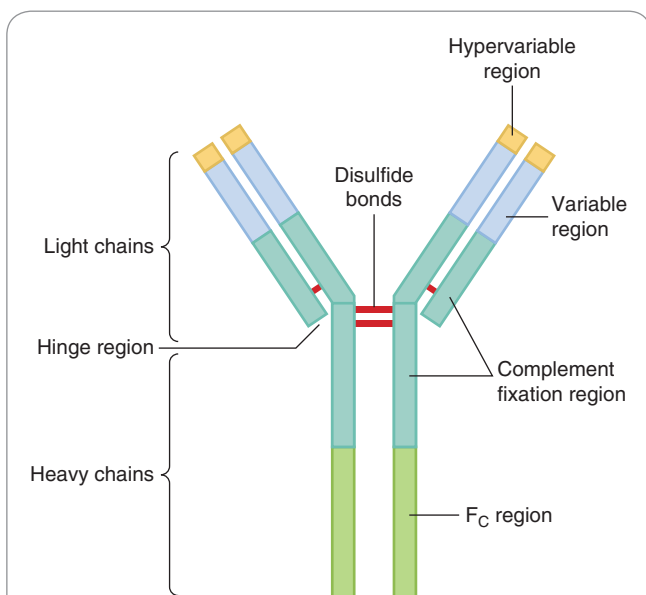
Fig. 59.1 Production of engineered 'chimeric' and 'humanised' monoclonal antibodies. The Y-shaped antibody molecule consists of two main domains: the Fc (constant) domain and the Fab (antibody-binding) domain. At the tip of the Fab regions (on the arms of the 'Y') are the hypervariable regions that actually bind the antigen. Chimeric antibodies are produced by replacing the murine Fc region with its human equivalent by altering and splicing the gene. For humanised antibodies, only the murine hypervariable regions are retained, the remainder of the molecule being human in origin. *(After Walsh, 2004.)*

The pharmacology of biopharmaceuticals is complicated because they may have multiple or even unknown modes of action, and partly because of their complex drug-receptor interactions many exhibit non-linear log dose-response curves. Erythropoietin, for example, has a bell-shaped dose response and, in the case of many monoclonal antibodies, there is a single optimal biological dose instead of the proportional effects that we are more accustomed to when dealing with small molecule drugs. Their pharmacokinetics are different too. We can no longer rely on the concepts such as Phase 1 and Phase 2 metabolism (see Ch. 9) to predict how they will be eliminated. Proteolytic degradation is more likely to be the important route for removal.

Whilst it is possible for generic manufacturers to copy conventional small molecule drugs when these come off patent, this cannot be done with biopharmaceuticals, which may depend upon the unique properties of a particular proprietary construct or clone. This means that *biosimilars*, as they are known in the argot of the industry, may not always have the same pharmacology as the original: obviously a big problem for regulators.

GENE THERAPY

Despite high hopes and intensive research efforts since the 1980s, realisation of the potential of gene therapy is still in its infancy. Here we focus first on the main problems and approaches being tried, with a final section on the limited success so far.

GENE DELIVERY

The transfer of recombinant nucleic acid into target cells – a special instance of the 'drug distribution' problem – is critical to the success of gene therapy. The constructs must pass from the extracellular space across the plasma and nuclear membranes, and be incorporated into the chromosomes. Because DNA is negatively charged and single genes have molecular weights around 10^4 times greater than conventional drugs, the problem is of a different order from the equivalent stage of routine drug development.

There are several important considerations in choosing a delivery system; these include:

- the *capacity* of the system (e.g. how much DNA it can carry)
- the *transfection efficiency* (its ability to enter and become utilised by cells)
- the *lifetime* of the transfected material (determined by the lifetime of the targeted cells)
- the *safety issue*, especially important in the case of viral delivery systems.

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Table 59.3 Characteristics of some delivery systems for gene therapy

Vector	Advantages	Disadvantages	Utilisation of system*
Liposomes	Virus-free, cheap to produce	Low efficiency, sometimes cytotoxic	6%
DNA cassettes	Virus-free	Low efficiency, expression temporary	18%
Herpes simplex virus type I	Highly infective, persistent expression	No integration with host DNA, cytotoxic, difficult to handle	3%
Adenovirus	Highly infective in epithelia	Immunogenic and transient, requires repeated administration	23%
Adeno-associated virus	Stable	Low capacity	5%
Retrovirus	Efficient, permanent	Low capacity, unstable, must integrate into host DNA, requires dividing cells	22%

*The approximate percentage of trials employing this type of delivery system.
After Wolf & Jenkins 2002 and with data from Wirth et al. 2013.

Various approaches have been developed (see Table 59.3) in an attempt to produce the optimal system.

There are two main strategies for delivering genes into patients. Using the *in vivo strategy*, the vector containing the therapeutic gene is injected into the patient, either intravenously (in which case some form of organ or tissue targeting is required) or directly into the target tissue (e.g. the retina). The *ex vivo strategy* is to remove cells from the patient (e.g. stem cells from bone marrow or circulating blood, or myoblasts from a biopsy of striated muscle), treat them with the vector in the laboratory and inject the genetically altered cells back into the patient.

An ideal vector should be *safe*, highly *efficient* (i.e. insert the therapeutic gene into a high proportion of target cells) and *selective* in that it should lead to expression of the therapeutic protein in the target cells but *not* to the expression of other viral proteins. Provided that the cell into which it is inserted is itself long-lived, the vector should ideally cause persistent expression, avoiding the need for repeated treatment. The latter consideration can be a problem in some tissues. In the autosomal recessive disorder *cystic fibrosis*, for example, the airway epithelium malfunctions because it lacks a membrane Cl⁻ transporter known as the *cystic fibrosis transport regulator* (CFTR). Epithelial cells in the airways are continuously dying and being replaced, so even if the CFTR gene were stably transfected into the epithelium, there would still be a periodic need for further treatment unless the gene could be inserted into the progenitor (stem) cells. Similar problems are anticipated in other cells that turn over continuously, such as gastrointestinal epithelium and skin.

VIRAL VECTORS

▼ Many contemporary gene delivery strategies aim to capitalise on the capacity of viruses to subvert the transcriptional machinery of the cells they infect and their ability (in some cases) to fuse with the host genome. While seemingly simple, there remain substantial practical problems with the *viral vector* approach. As viruses have evolved the means to invade human cells, so humans have evolved immune responses and other protective counter-measures. Although irritating in some respects, this is not all bad news from the point of view of safety. As many of the viruses used for vectors are pathogenic, they are usually modified such that they are 'replication defective' to avoid toxicity.

Retroviruses

▼ If introduced into stem cells, retroviral vectors have the attraction that their effects are persistent because they are incorporated into, and replicate with, host DNA, and so the 'therapeutic' gene is passed down to each daughter cell during division. Against this, the *retroviral integrase* randomly inserts the construct into chromosomes, so it may cause damage. Also, retroviruses could infect germ or non-target cells and produce undesired effects if administered *in vivo*. For this reason, retroviruses have been used mainly for *ex vivo* gene therapy. The life cycle of naturally occurring retroviruses may be exploited to create useful vectors for gene therapy (see Fig. 59.2).

Many viruses are equipped to infect specific cell types, though not necessarily the target cell of interest. It is possible to alter the retroviral envelope to alter specificity, such that the vector could be administered systemically but would target only the desired cell population. An example of this approach with a *lentivirus* (a type of retrovirus) is the substitution of the envelope protein of a non-pathogenic vector (e.g. mouse leukaemia virus) with the envelope protein of human vesicular stomatitis virus, in order specifically to target human epithelial cells.

Most retrovirus vectors are unable to penetrate the nuclear envelope, and because the nuclear membrane dissolves during cell division, they only infect dividing cells rather than non-dividing cells (such as adult neurons).

Adenovirus

▼ Adenovirus vectors are popular because of the high transgene expression that can be achieved. They transfer genes to the nucleus of the host cell, but (unlike retroviruses) these are not inserted into the host genome and so do not produce effects that outlast the lifetime of the transfected cell. This property also obviates the risk of disturbing the function of other cellular genes and the theoretical risks of carcinogenicity and germ cell transfection. Because of these favourable properties, adenovirus vectors have been used for *in vivo* gene therapy. Engineered deletions in the viral genome, render it unable to replicate or to cause widespread infection in the host while at the same time creating space in the viral genome for the therapeutic transgene to be inserted.

One of the first adenoviral vectors lacked part of a growth-controlling region called E_1 while incorporating the desired transgene. This vector gave excellent results, demonstrating gene transfer to cell lines and animal models of disease, but it has been disappointing as a treatment for cystic fibrosis) in human trials. Low doses (administered by aerosol to patients with this disease) produced only a very low-efficiency transfer, whereas higher doses caused inflammation, a host immune response and short-lived gene expression. Furthermore, treatment could not be repeated because of neutralising antibodies. This has led to recent attempts to manipulate adenoviral vectors to mutate or remove the genes that are most strongly immunogenic.
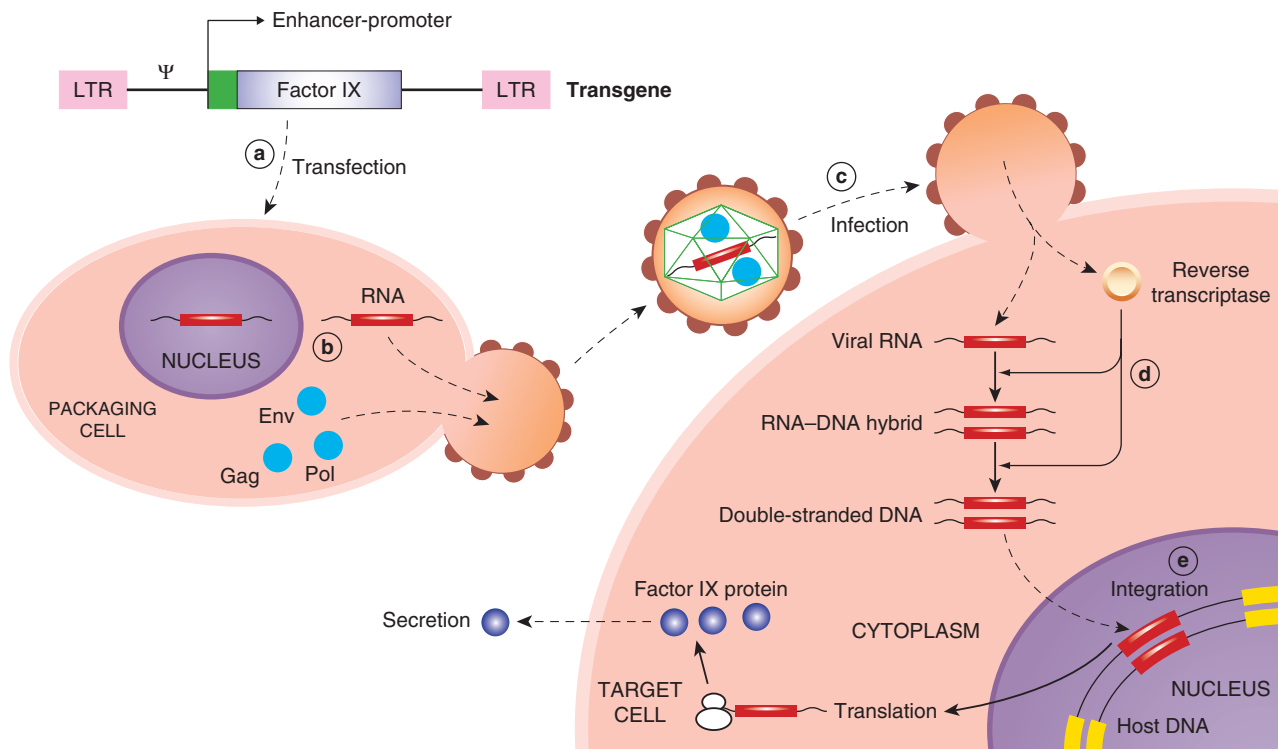
Fig. 59.2 Strategy for making retroviral vectors. The transgene (the example shows the gene for factor IX) in a vector backbone is introduced (a) into a packaging cell, where it is integrated into a chromosome in the nucleus, and (b) transcribed to make vector mRNA, which is packaged into the retroviral vector and shed from the packaging cell. It then infects the target cell (c). Virally encoded reverse transcriptase (d) converts vector RNA into an RNA–DNA hybrid, and then into double-stranded DNA, which is integrated (e) into the genome of the target cell. It can then be transcribed and translated to make factor IX protein. 'Env', Gag' and 'Pol' represent components of the retroviral vector. *(Redrawn from Verma, I.M., Somia, N., 1997. Gene therapy – promises, problems and prospects. Nature 389, 239–242.)*

Other viral vectors

▼ Other potential viral vectors under investigation include *adeno-associated virus*, *herpes virus* and disabled versions of *human immunode-ficiency virus* (HIV). Adeno-associated virus associates with host DNA but is not activated unless the cell is infected with an adenovirus. It is less immunogenic than other vectors but is difficult to mass produce and cannot be used to carry large transgenes. Herpes virus does not associate with host DNA but is very long lived in nervous tissue (so could have a specific application in treating neurological disease). HIV, unlike most other retroviruses, can infect non-dividing cells such as neurons. It is possible to remove the genes from HIV that control replication and substitute other genes. Alternatively, it may prove possible to transfer to other non-pathogenic retroviruses those genes that permit HIV to penetrate the nuclear envelope.

NON-VIRAL VECTORS

Liposomes

▼ Non-viral vectors include a variant of liposomes (Ch. 8). Plasmids (diameter up to approximately 2 μm) are too big to package in regular liposomes (diameter 0.025–0.1 μm), but larger particles can be made from positively charged lipids ('lipoplexes'), which interact with both negatively charged cell membranes and DNA, improving delivery into the cell nucleus and incorporation into the host chromosome. Such particles have been used to deliver the genes for HLA-B7, interleukin-2 and CFTR. They are much less efficient than viruses, and attempts are currently under way to improve this by incorporating various viral signal proteins (membrane fusion proteins, for example) in their outer coat. Direct injection of these complexes into solid tumours (e.g. melanoma, breast, kidney and colon cancers) can, however, achieve high local concentrations within the tumour.

Microspheres

▼ Biodegradable microspheres made from polyanhydride co-polymers of fumaric and sebacic acids (see Ch. 8) can be loaded with plasmid DNA. A plasmid with bacterial β-galactosidase activity formulated in this way and given by mouth to rats has resulted in systemic absorption and expression of the bacterial enzyme in the rat liver, raising the possibility of oral gene therapy.

Plasmid DNA

▼ Surprisingly, plasmid DNA itself ('naked DNA') enters the nucleus of some cells and is expressed, albeit much less efficiently than when it is packaged in a vector. Such DNA carries no risk of viral replication and is not usually immunogenic, but it cannot be targeted precisely. There is considerable interest in the possibility of using naked DNA for vaccines, because even very small amounts of foreign protein can stimulate an immune response. There are several theoretical advantages in this approach, numerous trials are taking place and several products have been licensed (Liu, 2011).

CONTROLLING GENE EXPRESSION

To realise the full potential of gene therapy, it is not enough to transfer the gene selectively to the desired target cells and maintain acceptable expression of its product – difficult though these goals are. It is also essential that the activity of the gene is controlled. Historically, it was the realisation of the magnitude of this task that diverted attention from the haemoglobinopathies (which were the first projected targets of gene therapy). Correction of these disorders demands an appropriate balance of normal α- and β-globin chain synthesis to be effective,

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and for this, and many other potential applications, precisely controlled gene expression will be essential.

▼ It has not yet proved possible to control transgenes precisely in human recipients, but there are techniques that may eventually enable us to achieve this goal. One hinges on the use of an inducible expression system. This is a fairly standard technique whereby the inserted gene also includes a **doxycycline**-inducible promoter such that expression of the gene can be switched on or off by treatment with, or withdrawal of, doxycycline.

The control of transfected genes is important in gene targeting as well. By splicing the gene of interest with a tissue-specific promoter, it should be possible to restrict expression of the gene to the target tissue. Such an approach has been used in the design of gene therapy constructs for use in ovarian cancer, the cells of which express several proteins at high abundance, including the proteinase inhibitor SLP1. In combination with the SLP1 promoter, plasmids carrying various genes were successfully and selectively expressed in ovarian cancer cell lines (Wolf & Jenkins, 2002).

SAFETY AND SOCIETAL ISSUES

Gene therapy tends to provoke deep unease in some sectors of society – witness the GM crop debate. Some of this reaction may be traced to ignorance or prejudice but it is nevertheless a problem that can hinder the introduction of new agents. Societal issues aside, the technique raises a number of specific concerns that generally relate to the use of viral vectors. These are usually selected because they are non-pathogenic, or modified to render them innocuous, but there is a concern that such agents might still acquire virulence during use. Retroviruses, which insert randomly into host DNA, could damage the genome and interfere with the protective mechanisms that normally regulate the cell cycle (see Ch. 5), and if they happen to disrupt essential cellular functions, this could increase the risk of malignancy.[6]

Another problem is that immunogenic viral proteins may be expressed that elicit an inflammatory response, and this could be harmful in some situations (e.g. in the airways of patients with cystic fibrosis). Initial clinical experience was reassuring, but the death of Jesse Gelsinger, an 18-year-old volunteer in a gene therapy trial for the non-fatal disease *ornithine decarboxylase deficiency* (which can be controlled by diet and drugs), led to the appreciation that safety concerns related to immune-mediated responses to vectors are very real (see Marshall, 1999).

THERAPEUTIC APPLICATIONS

Despite technical problems and safety concerns, there have been some encouraging successes. The European Medicines Agency granted its first license for a gene therapy product in 2012. **Glybera** is an adeno-associated virus construct that delivers a correct copy of lipoprotein lipase to patients lacking this enzyme (a very rare disorder, causing severe pancreatitis). Table 59.4 details some other examples and the area has been comprehensively reviewed by Wirth et al. (2013).

There are over 1800 gene therapy trials under way, recorded online at *Gene Therapy Review* (www.

genetherapyreview.com). Together with other resources (see Further Reading), this provides an enormous amount of relevant information. We conclude this section with a few comments on prominent applications of gene therapy.

GENE THERAPY FOR CANCER

Gene therapy for cancer and related diseases comprises almost 70% of all trials at the time of writing. Several therapeutic approaches (see Barar & Omidi, 2012) are under investigation, including:

Gene delivery and expression

- Gene delivery is one of the main hurdles to practical gene therapy.
- Recombinant genes are transferred using a vector, often a suitably modified virus.
- There are two main strategies for delivering genes into patients:
  - *in vivo* injection of the vector directly into the patient (e.g. into a malignant tumour)
  - *ex vivo* treatment of cells from the patient (e.g. stem cells from marrow or circulating blood), which are then returned to the patient.
- An ideal vector would be safe, efficient, selective and produce long-lasting expression of the therapeutic gene.
- Viral vectors include retroviruses, adenoviruses, adeno-associated virus, herpesvirus and disabled human immunodeficiency virus (HIV):
  - *retroviruses* infect many different types of dividing cells and become incorporated randomly into host DNA
  - *adenoviruses* are genetically modified to prevent replication and accommodate the therapeutic transgene. They transfer genes to the nucleus but not to the genome of the host cell. Problems include a strong host immune response, inflammation and short-lived expression. Treatment cannot be repeated because of neutralising antibodies
  - *adeno-associated virus* associates with host DNA and is non-immunogenic but is hard to mass-produce and has a small capacity
  - *herpesvirus* does not associate with host DNA but persists in nervous tissue and may be useful in treating neurological disease
  - disabled versions of HIV differ from most other retroviruses in that they infect non-dividing cells, including neurons.
- Non-viral vectors include:
  - a variant of liposomes, made using positively charged lipids and called 'lipoplexes'
  - biodegradable microspheres, which may offer orally active gene therapy
  - plasmid DNA ('naked DNA'), which can be used as a vaccine.
- A *tetracycline-inducible expression system* or similar technique can control the activity of the therapeutic gene.

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[6]This risk is more than a theoretical possibility; several children treated for *severe combined immunodeficiency* (SCID) with a retrovirus vector developed a leukaemia-like illness (Woods et al., 2006). The retroviral vector was shown to have inserted itself into a gene called *LMO-2*, mutations of which are associated with childhood cancers.

Table 59.4 Some gene therapy successes

Disease target	Gene delivered	Vector	Technique	Reference
X-linked severe combined immunodeficiency	IL2 cytokine receptor subunit gamma chain	Murine leukaemia retrovirus	Transfusion of patient with bone marrow cells transfected *ex vivo*	Hacein-Bey-Abina et al., 2010
Leber's congenital amaurosis	Isomerohydrolase retinal pigment epithelium protein	Adeno-associated virus	Subretinal injection	Maguire et al., 2009
Heart failure	Ca^{2+}-ATPase	Adeno-associated virus	Intracoronary infusion	Jessup et al., 2011
β-thalassaemia	β-globin	Lentivirus	Transfusion of patient with bone marrow cells transfected *ex vivo*	Cavezzana-Calvo et al., 2010
Metachromatic leukodystrophy	Arylsulfatase	Lentivirus	*Ex vivo* transfection of haematopoietic stem cells and transfusion into patient	Biffi et al., 2013
Wiskott–Aldrich syndrome	WAS protein	Lentivirus	*Ex vivo* transfection of haematopoietic stem cells and transfusion into patient	Aiuti et al., 2013

Safety

- There are those safety concerns that are specific to any particular therapy (e.g. polycythaemia from overexpression of **erythropoietin**) and additional general concerns relating, for example, to the nature of vectors.
- Viral vectors:
  – might acquire virulence during use
  – contain viral proteins, which may be immunogenic
  – can elicit an inflammatory response
  – could damage the host genome and interfere with the cell cycle, provoking malignancy.
- The limited clinical experience to date has not so far provided evidence of insurmountable problems.

Gene therapy for cancer

- Promising approaches include:
  – restoring protective proteins such as p53
  – inactivating oncogenes
  – delivering a gene to malignant cells that renders them sensitive to drugs
  – delivering a gene to healthy host cells to protect them from chemotherapy
  – tagging cancer cells with genes that make them immunogenic.

- restoring 'protective' proteins, such as the tumour suppressor gene (see Ch. 5)
- inactivating oncogene expression (e.g. by using a retroviral vector bearing an antisense transcript RNA to the *k-ras* oncogene)
- delivering a gene to malignant cells that renders them sensitive to cytotoxic drugs (e.g. thymidylate kinase, which activates **ganciclovir**) – the so-called 'suicide gene' approach
- delivery of proteins to healthy host cells in order to protect them (e.g. addition of the multidrug resistance channel to bone marrow cells *ex vivo*, thereby rendering them resistant to drugs used in chemotherapy)
- tagging cancer cells with genes expressing proteins that render malignant cells more visible to the immune system (e.g. for antigens such as HLA-B7 or cytokines such as granulocyte-macrophage colony-stimulating factor and interleukin-2).

SINGLE-GENE DEFECTS

Single-gene (*monogenic*) disorders were the obvious starting point for gene therapy trials and haemoglobinopathies were the first projected targets, but early attempts (in the 1980s) were put 'on hold' because of the problem (mentioned above) posed by the need to control precisely the expression of the genes encoding the different polypeptide chains of the haemoglobin molecule. Patients with thalassaemia (the commonest monogenic disease) exhibit enormous phenotypic diversity and hence variable clinical symptoms because, even in monogenic disorders, other genes as well as environmental factors are also important. However, some successes have recently been reported following transfection of bone marrow cells with a correct copy of the β-globin gene (see Table 59.4).

Another early target was cystic fibrosis, but progress here has been slow (see Prickett & Jain, 2013 for details). There have been other successes though. For example, *X-linked chronic granulomatous disease* has been successfully treated using a retroviral technique to deliver a functional version of the mutated NADPH oxidase protein (Ott et al., 2006 and Fig. 59.3) and a form of inherited blindness, *Leber's congenital amaurosis*, associated with a mutation in a gene that produces retinal pigment, has been rectified
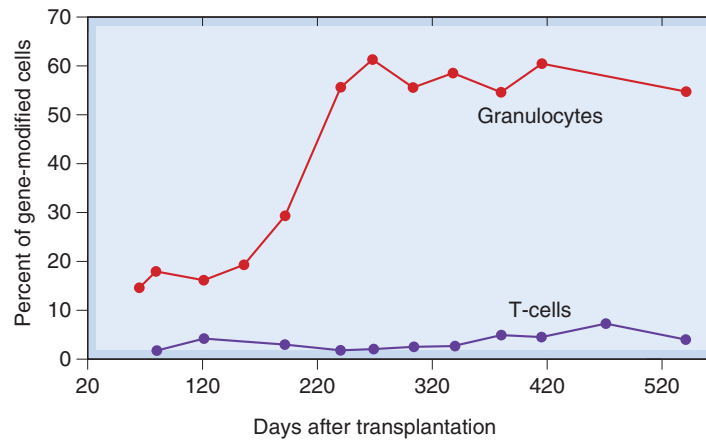
Fig. 59.3 Correcting an inherited defect using gene therapy. In this clinical trial, two patients with X-linked chronic granulomatous disease were transfused with GM–CSF-treated peripheral blood cells that had been genetically modified with a retroviral vector bearing the intact *gp91phox* gene ('*in vitro* protocol' – see text). The graph shows that the number of gene-modified peripheral blood leukocytes remained high for well over a year and this was accompanied by good levels of superoxide production in these cells – a clinical 'cure'. *(Data redrawn from Ott et al., 2006.)*

using an adeno-associated virus vector bearing a cDNA coding for the intact gene (Maguire et al., 2009).

GENE THERAPY AND INFECTIOUS DISEASE

In addition to DNA vaccines mentioned above, there is considerable interest in the potential of gene therapy for HIV infection. The aim is to render stem cells (which differentiate into immune cells) resistant to HIV before they mature. For an account of the strategies under investigation, see Chung et al. (2013).

GENE THERAPY AND CARDIOVASCULAR DISEASE

Gene therapy trials for treating cardiovascular diseases are reviewed by Bradshaw and Baker (2013). Vascular gene transfer is attractive not least because cardiologists and vascular surgeons routinely perform invasive studies that offer the opportunity to administer gene therapy vectors *ex vivo* (e.g. to a blood vessel that has been removed to use as an autograft) or locally *in vivo* (e.g. by injection through a catheter directly into a diseased coronary or femoral artery) (see Table 59.4). The nature of many vascular disorders, such as restenosis following angioplasty (stretching up a narrowed artery using a balloon that can be inflated via a catheter), is such that transient gene expression might be all that is needed therapeutically. Extension of vein graft patency by gene therapy approaches has been reviewed by Chandiwal et al. (2005). This is a promising area; for further details of angiogenic gene therapy see Hammond and McKirnan (2001) and of peripheral vascular disease, Ghosh et al. (2008).

OLIGONUCLEOTIDE APPROACHES

So far we have largely been considering the addition of entire genes, but there are other, related nucleic acid-based therapeutic strategies. Another approach is the use of *antisense oligonucleotides*. These are short (15–25 mer) oligonucleotides that are complementary to part of a gene or gene product that it is desired to inhibit. These snippets of genetic material can be designed to suppress the expression of a harmful gene either by forming a triplex (three-stranded helix) with a regulatory component of chromosomal DNA, or by complexing a region of mRNA. Oligonucleotides can cross plasma and nuclear membranes by endocytosis as well as by direct diffusion, despite their molecular size and charge. However, there are abundant enzymes that cleave foreign DNA in plasma and in cell cytoplasm, so enzyme-resistant *methylphosphorate* and *phosphothiorate* analogues have been developed. The oligomer needs to be at least 15 bases long to confer specificity and tight binding

Following parenteral administration, such oligomers distribute widely (although not to the CNS) and work in part by interfering with the transcription of mRNA and in part by stimulating its breakdown by ribonuclease H, which cleaves the bound mRNA. Mipomersen, a phosphothiorate analogue that suppresses the expression of apolipoprotein B, to be used for the treatment of a rare form of hypercholesterolaemia, is the first licensed antisense therapeutic, registered in the USA in 2013. This approach is being used in clinical studies in patients with viral disease (including HIV infection) and malignancy (including the use of Bcl-2 antisense therapy administered subcutaneously in patients with non-Hodgkin's lymphoma).

A related approach (see Castanatto & Rossi, 2009), which provides more efficient gene silencing than antisense oligonucleotides, is the use of *short interfering RNA* (siRNA),[7] whereby short lengths of double-stranded RNA recruit an enzyme complex, known as RISC, which selectively degrades the corresponding mRNA produced by the cell, thereby blocking expression. Clinical trials of siRNA therapeutics are in progress.

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[7]Discovered when it was found by plant scientists, to their surprise, that introducing RNA that encoded the colour-producing enzyme in petunias made the flowers less colourful, not more so. Subsequently siRNA has emerged as an important physiological mechanism for controlling gene expression, leading to the 2006 Nobel Prize award to Mello and Fire.

REFERENCES AND FURTHER READING

General reviews on biopharmaceuticals, gene therapy and utilities

Scientific American *published an issue devoted to gene therapy in June 1997, which is an excellent introduction, including articles by T. Friedmann ('Overcoming the obstacles to gene therapy'), P. L. Felgner (on non-viral strategies for gene therapy), R. M. Blaese (on gene therapy for cancer) and D. Y. Ho and R. M. Sapolsky (on gene therapy for the nervous system)*

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Useful Web resources

<www.genetherapynet.com> (*Gene Therapy Net – a fantastic resource for both patients and professionals. It is a veritable clearing house for information and up-to-date news on all aspects of gene therapy. It even advertises for volunteers and has a 'jobs' section, in case you are tempted! Has links to other related sites*)

60 Drug discovery and development

OVERVIEW

With the development of the pharmaceutical industry towards the end of the 19th century, drug discovery became a highly focused and managed process. Discovering new drugs moved from the domain of inventive doctors to that of scientists hired for the purpose. The bulk of modern therapeutics, and of modern pharmacology, is based on drugs that came from the laboratories of pharmaceutical companies, without which neither the practice of therapeutics nor the science of pharmacology would be more than a pale fragment of what they have become.

In this chapter we describe in outline the main stages of the process, namely (i) the discovery phase, i.e. the identification of a new chemical entity as a potential therapeutic agent; and (ii) the development phase, during which the compound is tested for safety and efficacy in one or more clinical indications, and suitable formulations and dosage forms devised. The aim is to achieve registration by one or more regulatory authorities, to allow the drug to be marketed legally as a medicine for human use.

Our account is necessarily brief and superficial, and more detail can be found elsewhere (Hill & Rang, 2013).

THE STAGES OF A PROJECT

Figure 60.1 shows in an idealised way the stages of a 'typical' project, aimed at producing a marketable drug that meets a particular medical need (e.g. to retard the progression of Parkinson's disease or cardiac failure, or to treat drug-resistant infections).

Broadly, the process can be divided into three main components:

1. *Drug discovery*, during which candidate molecules are chosen on the basis of their pharmacological properties.
2. *Preclinical development*, during which a wide range of non-human studies (e.g. toxicity testing, pharmacokinetic analysis and formulation) are performed.
3. *Clinical development*, during which the selected compound is tested for efficacy, side effects and potential dangers in volunteers and patients.

These phases do not necessarily follow in strict succession, as indicated in Figure 60.1, but generally overlap.

THE DRUG DISCOVERY PHASE

Given the task of planning a project to discover a new drug to treat – say, Parkinson's disease – where does one start? Assuming that we are looking for a novel drug rather than developing a slightly improved 'me-too' version of a drug already in use,[1] we first need to choose a new molecular target.
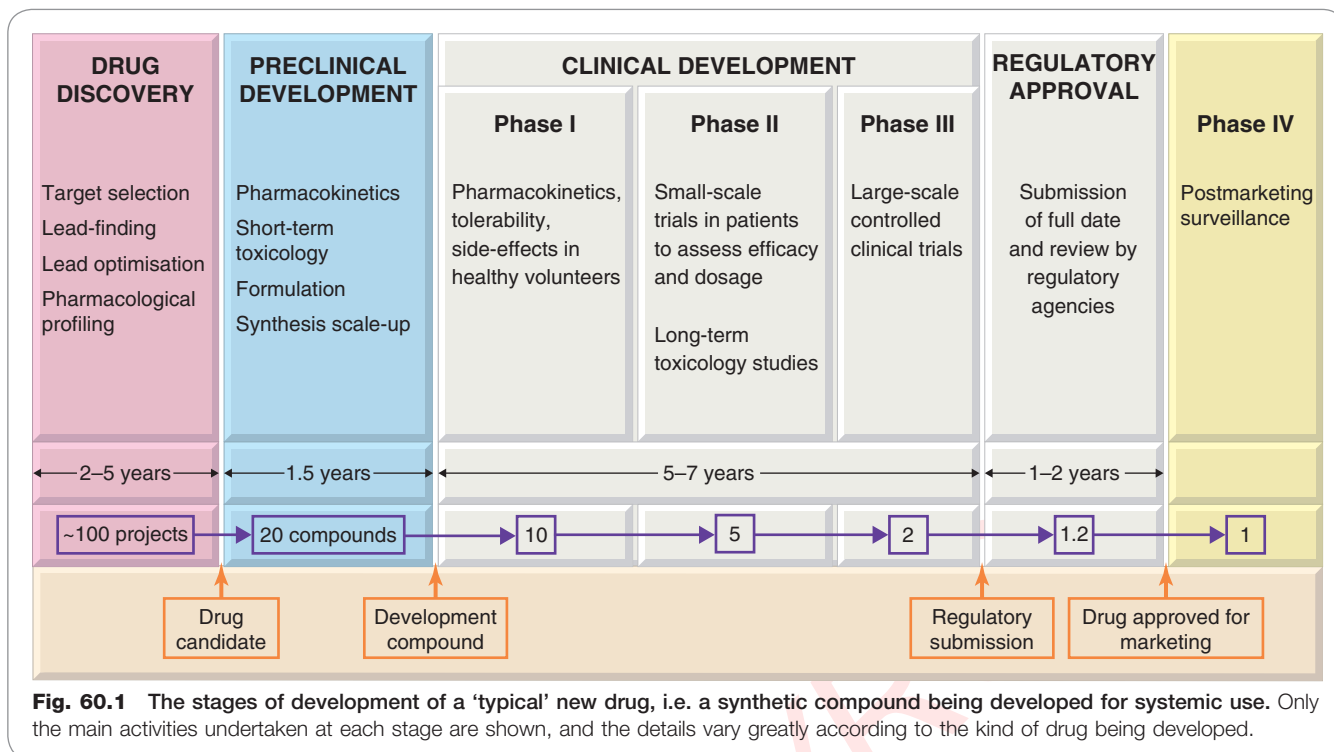
TARGET SELECTION

As discussed in Chapter 2, drug targets are currently, with few exceptions, functional proteins (e.g. receptors, enzymes, transport proteins). Although, in the past, drug discovery programmes were often based – successfully – on measuring a complex response *in vivo*, such as prevention of experimentally induced seizures, lowering of blood sugar or suppression of an inflammatory response, without the need for prior identification of a drug target, nowadays this is rare, and the first step is target identification. This most often comes from biological intelligence. It was known, for example, that inhibiting angiotensin-converting enzyme lowers blood pressure by suppressing angiotensin II formation, so it made sense to look for antagonists of the vascular angiotensin II receptor – hence the successful 'sartan' series of antihypertensive drugs (Ch. 22). Similarly, the knowledge that breast cancer is often oestrogen-sensitive led to the development of aromatase inhibitors such as **anastrozole**, which prevents oestrogen synthesis. Therapeutic drugs in use in 2005 addressed 266 distinct human targets (see Overington et al., 2006), but there are many proteins that are thought to play a role in disease for which we still have no cognate drug, and many of these represent potential starting points for drug discovery. Estimates range from a few hundred to several thousand potential drug targets that remain to be exploited therapeutically (see Betz, 2005). Selecting *valid* and '*druggable*' targets from this plethora is a major challenge.

Conventional biological wisdom, drawing on a rich fund of knowledge of disease mechanisms and chemical signalling pathways, coupled with genomic data, is the basis on which novel targets are most often chosen. Genomics is playing an increasing role by revealing new proteins involved in chemical signalling and new genes involved in disease. Space precludes discussion here of this burgeoning area; interested readers are referred to more detailed accounts (Lindsay, 2003; Kramer & Cohen, 2004; Semizarov & Blomme, 2008; Hill & Rang, 2013).

Overall, it is evident that in the foreseeable future there is ample biological scope in terms of novel drug targets for therapeutic innovation. What limits innovation is not the biology and primary pharmacology, but other factors, such as the emergence of unforeseen adverse effects during clinical testing, and the cost and complexity of drug discovery and development in relation to healthcare economics and increasing regulatory hurdles.

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[1]Many commercially successful drugs have in the past emerged from exactly such 'me-too' projects, examples being the dozen or so β-adrenoceptor-blocking drugs developed in the wake of propranolol, and the many 'triptans' that followed the introduction of sumatriptan to treat migraine. Quite small improvements (e.g. in pharmacokinetics or side effects), coupled with aggressive marketing, have often proved enough, but the barriers to registration are getting higher, so the emphasis has shifted towards developing innovative (first in class) drugs aimed at novel molecular targets.

Fig. 60.1 The stages of development of a 'typical' new drug, i.e. a synthetic compound being developed for systemic use. Only the main activities undertaken at each stage are shown, and the details vary greatly according to the kind of drug being developed.

LEAD FINDING

When the biochemical target has been decided and the feasibility of the project has been assessed, the next step is to find *lead compounds*. Commonly this involves cloning of the target protein – normally the human form, because the sequence variation among species is often associated with pharmacological differences, and it is essential to optimise for activity in humans. An assay system must then be developed, allowing the functional activity of the target protein to be measured. This could be a cell-free enzyme assay, a membrane-based binding assay or a cellular response assay. It must be engineered to run automatically, if possible with an optical read-out (e.g. fluorescence or optical absorbance), and in a miniaturised multiwell plate format for reasons of speed and economy. Robotically controlled assay facilities capable of testing tens of thousands of compounds per day in several parallel assays are now commonplace in the pharmaceutical industry, and have become the standard starting point for most drug discovery projects. For details on high-throughput screening, see Hüser (2006).

To keep such hungry monsters running requires very large compound libraries. Large companies will typically maintain a growing collection of a million or more synthetic compounds, which will be routinely screened whenever a new assay is set up. Whereas, in the past, compounds were generally synthesised and purified one by one, often taking a week or more for each, the use of combinatorial chemistry allows large families of related compounds to be made simultaneously. By coupling such high-speed chemistry to high-throughput assay systems, the time taken over the initial lead-finding stage of projects has been reduced to a few months in most cases, having previously often taken several years. Increasingly, use is being made of X-ray crystallography and other techniques to provide knowledge of the three-dimensional structure of the target protein and computer-based molecular modelling to generate possible lead structures within the compound library, in order to reduce the number of compounds to be screened. Refined in this way, screening is often successful in identifying lead compounds that have the appropriate pharmacological activity and are amenable to further chemical modification.

'Hits' detected in the initial screen often turn out to be molecules that have features undesirable in a drug, such as too high a molecular weight, excessive polarity and possession of groups known to be associated with toxicity. Computational 'prescreening' of compound libraries is often used to eliminate such compounds.

The hits identified from the primary screen are used as the basis for preparing sets of homologues by combinatorial chemistry so as to establish the critical structural features necessary for binding selectively to the target. Several such iterative cycles of synthesis and screening are usually needed to identify one or more lead compounds for the next stage.

Natural products as lead compounds

Historically, natural products, derived mainly from fungal and plant sources, have proved to be a fruitful source of new therapeutic agents, particularly in the field of anti-infective, anticancer and immunosuppressant drugs. Familiar examples include **penicillin**, **streptomycin** and many other antibiotics; vinca alkaloids; **paclitaxel**; **ciclosporin**; and **sirolimus** (**rapamycin**). These substances presumably serve a specific protective function, having evolved to recognise with great precision vulnerable target molecules in an organism's enemies or competitors. The surface of this resource has barely been scratched, and many companies are actively engaged in generating and testing natural product libraries for lead-finding purposes. Fungi and other microorganisms are particularly suitable for this, because they are ubiquitous, highly

diverse and easy to collect and grow in the laboratory. Compounds obtained from plants, animals or marine organisms are much more troublesome to produce commercially. The main disadvantage of natural products as lead compounds is that they are often complex molecules that are difficult to synthesise or modify by conventional synthetic chemistry, so that lead optimisation may be difficult and commercial production very expensive.

LEAD OPTIMISATION

Lead compounds found by random screening are the basis for the next stage, lead optimisation, where the aim (usually) is to increase the potency of the compound on its target and to optimise it with respect to other characteristics, such as selectivity and pharmacokinetic properties. In this phase, the tests applied include a broader range of assays on different test systems, including studies to measure the activity and time course of the compounds *in vivo* (where possible in animal models mimicking aspects of the clinical condition; see Ch. 7), and checking for unwanted effects in animals, evidence of genotoxicity and usually for oral absorption. The objective of the lead optimisation phase is to identify one or more *drug candidates* suitable for further development.

As shown in Figure 60.1, only about one project in five succeeds in generating a drug candidate, and it can take up to 5 years. The most common problem is that lead optimisation proves to be impossible; despite much ingenious and back-breaking chemistry, the lead compounds, like antisocial teenagers, refuse to give up their bad habits. In other cases, the compounds, although they produce the desired effects on the target molecule and have no other obvious defects, fail to produce the expected effects in animal models of the disease, implying that the target is probably not a good one. The virtuous minority proceed to the next phase, preclinical development.

PRECLINICAL DEVELOPMENT

The aim of preclinical development is to satisfy all the requirements that have to be met before a new compound is deemed ready to be tested for the first time in humans. The work falls into four main categories:

1. Pharmacological testing to check that the drug does not produce any obviously hazardous acute effects, such as bronchoconstriction, cardiac dysrhythmias, blood pressure changes and ataxia. This is termed *safety pharmacology*.
2. Preliminary toxicological testing to eliminate genotoxicity and to determine the maximum non-toxic dose of the drug (usually when given daily for 28 days, and tested in two species). As well as being checked regularly for weight loss and other gross changes, the animals so treated are examined minutely *post mortem* at the end of the experiment to look for histological and biochemical evidence of tissue damage (see also Ch. 57).
3. Pharmacokinetic testing, including studies on the absorption, metabolism, distribution and elimination (ADME studies) in the species of laboratory animals used for toxicology testing, so as to link the pharmacological and toxicological effects to plasma concentration and drug exposure.
4. Chemical and pharmaceutical development to assess the feasibility of large-scale synthesis and purification,

to assess the stability of the compound under various conditions and to develop a formulation suitable for clinical studies.

Much of the work of preclinical development, especially that relating to safety issues, is done under a formal operating code, known as *Good Laboratory Practice* (GLP), which covers such aspects as record-keeping procedures, data analysis, instrument calibration and staff training. The aim of GLP is to eliminate human error as far as possible, and to ensure the reliability of the data submitted to the regulatory authority, and laboratories are regularly monitored for compliance to GLP standards. The strict discipline involved in working to this code is generally ill-suited to the creative research needed in the earlier stages of drug discovery, so GLP standards are not usually adopted until projects get beyond the discovery phase.

Roughly half the compounds identified as drug candidates fail during the preclinical development phase; for the rest, a detailed dossier (the 'investigator brochure') is prepared for submission alongside specific study protocols to the regulatory authority such as the European Medicines Evaluation Agency or the US Food and Drugs Administration, whose permission is required to proceed with studies in humans. This is not lightly given, and the regulatory authority may refuse permission or require further work to be done before giving approval.

Non-clinical development work continues throughout the clinical trials period, when much more data, particularly in relation to long-term and reproductive toxicity in animals, has to be generated. Failure of a compound at this stage is very costly, and considerable efforts are made to eliminate potentially toxic compounds much earlier in the drug discovery process by the use of *in vitro*, or even *in silico*, methods.

CLINICAL DEVELOPMENT

Clinical development proceeds through four distinct but overlapping phases of clinical trials (see Ch. 7). For detailed information, see Friedman et al. (2010).

- *Phase I studies* are performed on a small group (normally 20–80) of volunteers – often healthy young men but sometimes patients, and their aim is to check for signs of any potentially *dangerous effects*, for example on cardiovascular,[2] respiratory, hepatic or renal function; *tolerability* (does the drug produce any unpleasant symptoms, for example headache, nausea, drowsiness?); and *pharmacokinetic properties* (is the drug well absorbed? Is absorption affected by food? What is the time course of the plasma concentration? Is there evidence of cumulation or non-linear kinetics?). Phase I studies may also test for pharmacodynamic effects in volunteers, sometimes called 'proof-of-concept' studies (e.g. does a novel analgesic compound block experimentally induced pain in humans? How does the effect vary with dose?).
- *Phase II studies* are performed on groups of patients (normally 100–300) and are designed to determine pharmacodynamic effect in patients, and if this is confirmed, to establish the dose regimen to be used in the definitive phase III study. Often, such studies

[2]QT prolongation, a sign of potentially dangerous cardiac arrhythmias (see Ch. 21), is a common cause of failure in early development, and regulators demand extensive – and expensive – studies to test for this risk.

will cover several distinct clinical disorders (e.g. depression, anxiety states and phobias) to identify the possible therapeutic indications for the new compound and the dose required. When new drug targets are being studied, it is not until these phase II trials are completed that the team finds out whether or not its initial hypothesis was correct, and lack of the expected effect is a common reason for failure.

- *Phase III studies* are the definitive double-blind, randomised trials, commonly performed as multicentre trials on thousands of patients, aimed at comparing the new drug with commonly used alternatives. These are extremely costly, difficult to organise and often take years to complete, particularly if the treatment is designed to retard the progression of a chronic disease. It is not uncommon for a drug that seemed highly effective in the limited patient groups tested in phase II to look much less impressive under the more rigorous conditions of phase III trials.

▼ The conduct of trials has to comply with an elaborate code known as Good Clinical Practice, covering every detail of the patient group, data collection methods, recording of information, statistical analysis and documentation.[3]

Increasingly, phase III trials are being required to include a *pharmacoeconomic analysis* (see Ch. 1), such that not only clinical but also economic benefits of the new treatment are assessed.

At the end of phase III, the drug will be submitted to the relevant regulatory authority for licensing. The dossier required for this is a massive and detailed compilation of preclinical and clinical data. Evaluation by the regulatory authority normally takes a year or more, and further delays often arise when aspects of the submission have to be clarified or more data are required. Eventually, about two-thirds of submissions gain marketing approval. Overall, only 11.5% of compounds entering Phase I are eventually approved (see Munos, 2009). Increasing this proportion by better compound selection at the laboratory stage is one of the main challenges for the pharmaceutical industry.

- *Phase IV studies* comprise the obligatory postmarketing surveillance designed to detect any rare or long-term adverse effects resulting from the use of the drug in a clinical setting in many thousands of patients. Such events may necessitate limiting the use of the drug to particular patient groups, or even withdrawal of the drug.[4]

BIOPHARMACEUTICALS

'Biopharmaceuticals', i.e. therapeutic agents produced by biotechnology rather than conventional synthetic chemistry, are discussed in Chapter 59. Such therapeutic agents comprise an increasing proportion – currently about 30% – of new products registered each year. The principles underlying the development and testing of biopharmaceuticals are basically the same as for synthetic drugs. In practice, biopharmaceuticals generally run into fewer toxicological problems than synthetic drugs,[5] but more

problems relating to production, quality control, immunogenicity and drug delivery. Walsh (2003) covers this specialised field in more detail.

COMMERCIAL ASPECTS

Figure 60.1 shows the approximate time taken for such a project and the attrition rate (at each stage and overall) based on recent data from several large pharmaceutical companies. The key messages are (i) that it is a high-risk business, with only about one drug discovery project in 50 reaching its goal of putting a new drug on the market, (ii) that it takes a long time – about 12 years on average and (iii) that it costs a lot of money to develop one drug (estimated at a mind-boggling $3.9 billion in 2008, see Munos, 2009).[6] For any one project, the costs escalate rapidly as development proceeds, phase III trials and long-term toxicology studies being particularly expensive. The time factor is crucial, because the new drug has to be patented, usually at the end of the discovery phase, and the period of exclusivity (20 years in most countries) during which the company is free from competition in the market starts on that date. After 20 years, the patent expires, and other companies, which have not supported the development costs, are free to make and sell the drug much more cheaply, so the revenues for the original company decrease rapidly thereafter. Many profitable drugs have come or are coming to the end of their patents between 2010 and 2015, adding to the industry's problems. Reducing the development time after patenting is a major concern for all companies, but so far it has remained stubbornly fixed at around 10 years, partly because the regulatory authorities are demanding more clinical data before they will grant a licence. In practice, only about one drug in three that goes on the market brings in enough revenue to cover its development costs. Success for the company relies on this one drug generating enough profit to pay for the rest.[7]

FUTURE PROSPECTS

Since about 1990, the drug discovery process has been in the throes of a substantial methodological revolution, following the rapid ascendancy of molecular biology, genomics and informatics, amid high expectations that this would bring remarkable dividends in terms of speed, cost and success rate. High-throughput screening has undoubtedly emerged as a powerful lead-finding technology, but overall the benefits are not yet clear: costs have risen steadily, the success rate has not improved (Fig. 60.2) and development times have not decreased.

Figure 60.2 illustrates the trend in the number of new drugs launched in the major markets worldwide, which has declined despite escalating costs and improved technology. There has been much speculation as to the causes, the optimistic view (see below) being that fewer but better

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[3]Similar strict codes must be followed in laboratory tests to determine safety (Good Laboratory Practice; see text) and drug manufacture (Good Manufacturing Practice).
[4]Recent high-profile cases include the withdrawal of rofecoxib (a cyclo-oxygenase-2 inhibitor; see Ch. 26) when it was found (in a Phase III trial for a new indication) to increase the frequency of heart attacks, and of cerivastatin (Ch. 23), a cholesterol-lowering drug found to cause severe muscle damage in a few patients.

[5]The serious toxicity caused to human volunteers in the 2006 phase I trials of the monoclonal antibody TGN 1412 (see Ch. 59) showed that this could not be relied on, and has led to substantial tightening of standards (and slowdown of the development of biopharmaceuticals).
[6]These cost estimates have been strongly challenged by commentators (see Angell, 2004) who argue that the pharmaceutical companies overestimate their costs several-fold in order to justify high drug prices.
[7]Actually, companies spend about twice as much on marketing and administration as on research and development.
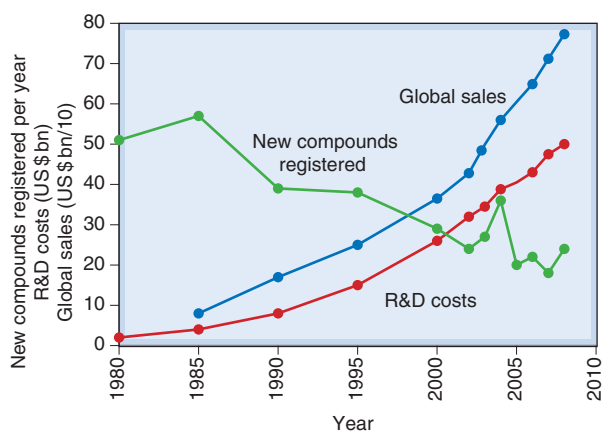
Fig. 60.2 **Research and development (R&D) spend, sales and new drug registrations, 1980–2009.** Registrations refer to new chemical entities (including biopharmaceuticals, excluding new formulations and combinations of existing registered compounds). The decline in registrations up to 2009 has since been halted (32 in 2012). *(Data from various sources, including the Centre for Medicines Research, Pharmaceutical Research and Manufacturers Association of America.)*

drugs are being introduced, and that the genomics revolution has yet to make its impact.

If the new drugs that are being developed improve the quality of medical care, there is room for optimism. In recent ('pre-revolutionary') years, synthetic drugs aimed at new targets (e.g. selective serotonin reuptake inhibitors, statins, kinase inhibitors and several monoclonal antibodies) have made major contributions to patient care. Even if the new technologies do not improve productivity, we can reasonably expect that their ability to make new targets available to the drug discovery machine will have a real effect on patient care. The decline in annual registrations between 1980 and 2000 (Fig. 60.2) has stabilised in the last decade, and even shows signs of an upturn more recently. Creativity remains high, despite the rising costs and declining profits that are a challenge to the pharmaceutical industry.

Trends to watch include the growing armoury of biopharmaceuticals, particularly monoclonal antibodies such as **trastuzumab** (an antibody directed against human epidermal growth factor receptor-2 – HER2 – which is used to treat breast cancers that overexpress this receptor) and **infliximab** (a tumour necrosis factor antibody used to treat inflammatory disorders; see Ch. 26); these are successful recent examples, and more are in the pipeline. Another likely change will be the use of genotyping to 'individualise' drug treatments, so as to reduce the likelihood of administering drugs to 'non-responders' (see Ch. 11, which summarises the current status of 'personalised medicine'). The implications for drug discovery will be profound, for the resulting therapeutic compartmentation of the patient population will mean that markets will decrease, bringing to an end the reliance on the 'blockbusters' referred to earlier. At the same time, clinical trials will become more complex (and expensive), as different genotypic groups will have to be included in the trial design. The hope is that therapeutic efficacy will be improved, not that it will be a route to developing drugs more cheaply and quickly. However, there is general agreement that the current modus operandi is commercially unsustainable (see Munos, 2009). Costs and regulatory requirements are continuing to rise, and the anticipated use of genomics to define subgroups of patients likely to respond to particular therapeutic agents (see Ch. 11) will mean fragmentation of the market, as we move away from the 'one-drug-suits-all' approach that encouraged companies to focus their efforts on blockbuster drugs. More niche products targeted at smaller patient groups will be needed, though each costs as much to develop as a blockbuster and carries a similar risk of failure.

A FINAL WORD

The pharmaceutical industry in recent years has attracted much adverse publicity, some of it well deserved, concerning drug pricing and profits, non-disclosure of adverse clinical trials data, reluctance to address major global heath problems such as tuberculosis and malaria, aggressive marketing practices and much else (see Angell, 2004; Goldacre, 2012). It needs to be remembered though that, despite its faults, the industry has been responsible for most of the therapeutic advances of the past half-century, without which medical care would effectively have stood still.

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