# Medicinal Chemistry of Anticancer Drugs

# Medicinal Chemistry of Anticancer Drugs

**Second Edition**

By

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# Foreword

Cancer can be considered a general term that covers over 200 malignancies different in their genetic basis, etiology, patterns of progression, and final clinical outcome. These pathogenic conditions are characterized by uncontrolled cellular proliferation and growth and, under some physiological conditions, spread to adjacent or remote organs and tissues.

We know now that cancer is a multifactorial disease, in which both environmental and genetic factors can transform normal cells into cancerous ones by altering the normal function of a wide spectrum of biological networks. The complexity of the underlying mechanisms of the biology of human cancer, in particular the multiple mutations that occur in oncogenes, tumor suppressor, or DNA repair genes, represents a major challenge in the identification and development of effective, durable, and safe cancer therapies.

The 15 chapters of "Medicinal Chemistry of Anticancer Drugs" provide a comprehensive overview of the different synthetic and biological approaches that have been attempted to interfere with cancer progression and, eventually, prevention (Chapter 15). The mechanisms of action of standard-of-care and novel anti-cancer drugs are broad and expand from the initial antimetabolites (Chapter 2), hormonal therapies (Chapter 3), radio- and photo-sensitizing agents (Chapter 4), DNA-interactive molecules (Chapters 5, 6, and 7), or tubulin inhibitors (Chapter 8) to the most recent-targeted therapeutic agents, which inhibit intracellular components of deregulated signal transduction, apoptotic, metastatic, or epigenetic processes (Chapters 9, 10, and 11).

One of the main issues faced by oncologists with the preceding agents is the development of drug resistance. To delay the onset of this problem, compounds that block some of the underlying mechanisms of resistance or are active against mutations in the kinase gene that abrogate drug binding have been identified and pursued in the clinic (Chapter 14). As shown in different parts of the book, combinations of drugs, including the use of alternative dosage regimes, are often required to maximize clinical benefit for the cancer patient.

In addition to chemotherapeutic agents, which for many years have been the backbone of numerous regimes for the treatment of solid and liquid tumors, a deeper understanding of the molecular events leading to tumor formation, invasion, angiogenesis, and metastasis and, more recently, the ability to boost the body's immune system have been exploited to advance new therapeutic modalities (e.g., humanized monoclonal antibodies, gene therapy, or antisense oligonucleotides; Chapter 12) or delivery systems (e.g. nanoparticles, liposomes, or polymer conjugates; Chapter 13) to achieve sustained responses and minimize systemic toxicity.

While many disappointments have been harvested in pre- and clinical settings, we start to witness the incremental improvements in survival achieved with the current armamentarium of anticancer drugs. Thus, recent epidemiological data provided by the American Cancer Society show that from 2006 to 2010, the death rate for all cancers combined decreased by 1.8% and 1.4% per year in men and women, respectively. These results are encouraging, provide hope to cancer patients and their families, and demonstrate that we are in the right path to achieve our ultimate objective: *to cure cancer*.

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**Sanofi**

# Preface

Chemistry, and medicinal chemistry in particular, is a very broad subject that bears a profound relationship with all phases of drug discovery, design, and development. The involvement of many facets of chemistry is needed for the translation of the knowledge of the cellular, molecular, and genetic basis of cancer into effective therapies. In the past decades, the boundaries between biology and chemistry have become increasingly diffuse because biology is close to becoming a chemical science. Indeed, it can be easily verified that in the past years many Nobel prizes in chemistry have been awarded for discoveries that are biological in their nature or applications. As our understanding of the basic chemistry of life increases, we begin to understand complex phenomena at molecular levels, and this level of understanding allows for the design of molecular entities that are selectively suited to interact with a given biological target because drug action is always a consequence of a chemical interaction.

As when the first edition of this book was planned, we believe that there is a clear need for an updated monograph of anticancer drugs from the point of view of medicinal chemistry. We have attempted to produce a concise but reasonably comprehensive treatment to fill the gap between the elementary medicinal chemistry textbooks and the primary literature and help readers to achieve a deeper understanding of the molecular basis of the action of antitumor drugs. Because of the huge number of agents that show *in vitro* antitumor activity, we had to make some difficult decisions on the inclusion or exclusion of certain topics and, with some exceptions related to recently validated targets, we have limited our discussion to agents that have been approved or at least have entered clinical trials. The organization of the book is based on targets and mechanisms of action using the main mechanism of action of each drug as the criterion, although some decisions taken in this regard might be debatable. We have purposefully excluded the discussion of antitumor drug synthesis, not because we believe that it is not pertaining in a book devoted to medicinal chemistry but because it would have required a full volume in itself to do some justice to the achievements made in this area.

In the past years, anticancer therapy has continued to be a very active field of research and, in addition to the large number of validated targets and new drugs that have been developed, a huge amount of knowledge has been generated, mainly based on genomic data and the understanding of cancer as a multifactorial disease. Modern anticancer drug research has become increasingly focused on signal transduction therapy, and many of the validated targets are transduction-related macromolecules. The development of specific monoclonal antibodies targeted at tumor antigens has undergone very fast growth. Drugs that interact with proteolytic enzymes involved in the proteasome machinery and in angiogenesis and metastasis, as well as drugs targeting epigenome alterations, also have much current relevance. Active immunotherapy (therapeutic vaccines), immunomodulators of the tumor environment, and nanotechnology approaches are innovative therapies for personalized treatments, which are expected to have a major clinical impact in the treatment of cancer.

The second edition of this book contains many new features. We have undertaken a thorough update of the text to include new drugs that have been introduced in recent years. In each chapter, we emphasize the basis of drug discovery and design, analyzing the problems found in their development, and updating the information on their clinical applications. We now include drug trade names in the belief that they will make the book more practical. We hope that the introduction of color in the figures and a large number of three-dimensional structures of drug–target complexes generated from Protein Data

Bank files leads to a clearer explanation of many aspects of the mechanisms discussed in the text and generally improves the reader's experience.

All chapters have been thoroughly rewritten and updated with discussion of many new targets and drugs, which has required the creation of a separate chapter for epigenetic cancer therapy (Chapter 8). A much-expanded treatment of targeted anticancer therapy by small molecules is provided in Chapters 10 and 11, and drugs acting on a large number of new targets are discussed. These include hepatocyte growth factor receptor (HGFR), fibroblast growth factor receptor (FGFR), anaplastic lymphoma kinase (ALK), JAK-STAT and PRL pathways, tropomyosin receptor kinase (Trk), Bruton's tyrosine kinase (BTK), checkpoint kinases (CHKs), Pim kinases, transforming growth factor-β (TGF-β)-Smad, NEDD 8 activating enzyme (NAE), anaphase-promoting complex (APC), aminopeptidase N, cathepsin, and integrins. New sections are devoted to several topics of current interest in this area, including drugs targeting cancer stem cells acting on the wingless/β-catenin (Wnt//β-Cat), Notch, and Hedgehog signaling pathways and drugs that interfere with oncogenic protein–protein interactions, with special emphasis on anticancer drugs acting on apoptotic signaling pathways. Inhibitors of kinases involved in anaerobic glycolysis are also discussed. Another new chapter dealing with biological cancer therapy has been introduced (Chapter 12), comprising topics such as monoclonal antibodies, cancer immunotherapy, cancer vaccines, cancer gene therapy, and antisense oligonucleotides. Chapter 13, devoted to methods for the specific delivery of anticancer drugs to tumors, has been much expanded, and approximately half of this material is now devoted to nanotechnologies. The chapter devoted to anticancer drug resistance (Chapter 14) now includes a discussion of cellular adhesion molecules and SPARC protein as mechanisms of resistance. Cancer chemoprevention (Chapter 15) has also been updated and expanded.

We expect that this book will be useful to undergraduate and postgraduate students of medicinal chemistry and their instructors, in courses related to pharmacy, chemistry, or the health sciences, and should also have some appeal for students of pharmacology or biochemistry courses. We also hope that the inclusion of a large number of references to the review and primary literature will also make the book useful for researchers and practitioners of health professions.

**Carmen Avendaño**
**José Carlos Menéndez**
**Madrid, January 2015**

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# Abbreviations

| | |
|---|---|
| ACAT | Acyl-coenzyme A: cholesterol acyl transferase |
| ACL | ATP-citrate lyase |
| ADC | Antibody–drug conjugate |
| ADCC | Antibody-mediated cellular cytotoxicity |
| ADEPT | Antibody-directed enzyme prodrug therapy |
| ADI-PEG20 | PEG-recombinant arginine deiminase |
| ADP | Adenosine diphosphate |
| AEBS | Antiestrogen binding site |
| AF | Activating function |
| AI | Aromatase inhibitor |
| AICARFT | Aminoimidazolecarboxamide ribonucleotide formyltransferase |
| AIDS | Acquired immunodeficiency syndrome |
| AIF | Apoptosis-inducing factor |
| ALDH | Aldehyde dehydrogenase |
| ALK | Anaplastic lymphoma kinase |
| ALK5 (TβR-I) | Activine-like kinase 5 |
| ALL | Acute lymphocytic leukemia |
| AML | Acute myeloid leukemia |
| AMP | Adenylic acid |
| AOL | Amine oxidase-like |
| AP-1 | Activator protein 1 |
| APC | Antigen-presenting cell |
| APC | Adenomatous polyposis coli |
| APC/C | Anaphase-promoting complex/cyclosome |
| APL | Acute promyelocytic leukemia |
| APN (CD13) | Aminopeptidase N |
| AR | Androgen receptor |
| ARCON | Accelerated radiotherapy with carbogen and nicotinamide |
| 5ARI | 5α-Reductase inhibitor |
| ASC carrier | Alanine, serine, and cysteine carrier |
| ASO | Antisense oligonucleotide |
| ATC | Anaplastic thyroid cancer |
| ATM | Ataxia telangiectasia mutated |
| TP | Adenosine triphosphate |
| ATRA | All-*trans* retinoic acid (tretinoin) |
| BCL2 | B-cell lymphoma protein 2 |
| BCR | Breakpoint cluster region |
| BER | Base excision repair |
| BET | Bromodomain and extra-terminal |
| BFC | Bifunctional chelate |
| BIR | Baculovirus IAP repeat |
| BLMs | Bleomycins |
| BNCT | Boron neutron capture therapy |
| BPA | Boronophenylalanine |
| BR | Binding region |
| *BRCA* | “Berkeley California” genes |

| | |
|---|---|
| BSH | Sodium borocaptate |
| BTK | Bruton's tyrosine kinase |
| BTZ | Benzotriazinyl |
| CAC | Cancer-associated cachexia |
| CaM | Calmodulin |
| CAM | Cell adhesion molecule |
| CBP | Cyclic AMP response element-binding protein |
| CD | Cluster of differentiation |
| CDK | Cyclin-dependent kinase |
| CDL | Cullin-dependent ligase |
| CDP | Cytidine diphosphate |
| CHK-1 | Checkpoint kinase 1 |
| CKI | CDK inhibitor |
| c-Met (HGFR) | Mesenchymal–epithelial transition factor |
| CML | Chronic myeloid leukemia |
| CNUs | 1-(2-Chloroethyl)-1-nitrosoureas |
| CR | Cisplatin resistant |
| CRD | Cysteine-rich domain |
| CRPC | Castration-resistant prostate cancer |
| CS | Cisplatin sensitive |
| CSC | Cancer stem cell |
| CSCC | Cholesterol side chain cleavage enzyme |
| CSNK1G3 | Casein kinase 1, gamma 3 |
| CtBP2 | C-terminal binding protein 2 |
| CTGF | Connective tissue growth factor |
| CTL | Cytotoxic T lymphocyte |
| Cts-L | Cathepsin L |
| cyt C | Cytochrome *c* |
| CYP | Cytochrome P |
| Dapk | Death-associated protein kinase |
| DASI | Dual aromatase–sulfatase inhibitor |
| DAUF | Daunoform |
| dFdC | Gemcitabine (difluorodeoxycytidine) |
| dG | Deoxyguanosine |
| DG | Diacylglycerol |
| 2-DG | 2-Deoxy-D-glucose |
| dGTP | Deoxyguanosine triphosphate |
| DHEA | Dehydroepiandrosterone |
| DHEA-S | dehydroepiandrosterone sulfate |
| DHF | Dihydrofolate |
| DHFR | Dihydrofolate reductase |
| DHFU | Dihydrofluorouracil |
| DHT | 5α-Dihydrotestosterone |
| DISC | Death-inducing signaling complex |
| DKK1 | Dickkopf-1 |
| DNA | Deoxyribonucleic acid |
| DNMT | DNA methyl transferase |
| DNR | Daunomycin |
| DNRol | Daunorubicinol |
| DOS | Diversity oriented synthesis |
| DOX | Doxorubicin (adriamycin) |
| DOXF | Doxoform |

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|---|---|
| DOXol | Doxorubicinol |
| DPD | Dihydropyrimidine dehydrogenase |
| DR | Death receptor |
| DSB | Double-strand break |
| DTD (NQO1) | DT-diaphorase |
| dTDP | Deoxythymidine diphosphate |
| dTMP | Deoxythymidine monophosphate |
| dTTP | Deoxythymidine triphosphate |
| dUMP | Deoxyuridine monophosphate |
| dUTP | Deoxyuridine triphosphate |
| $E_1$ | Estrone |
| $E_1S$ | Estrone sulfate |
| $E_2$ | Estradiol |
| $E_2$-1,2-Q | Estradiol-1,2-quinone |
| $E_2$-2,3-Q | Estradiol-2,3-quinone |
| $E_2$-3,4-Q | Estradiol-3,4-quinone |
| EBRT | External beam radiotherapy |
| EBV | Epstein–Barr virus |
| EGFR (HER-1) | Epidermal growth factor receptor |
| EML4 | Echinoderm microtubule-associated protein-like 4 |
| EMT | Epithelial–mesenchymal transdifferentiation |
| ENL | Erythema nodosum leprosum |
| EpCAM | Epithelial cell adhesion molecule |
| EPR | Enhanced permeability and retention |
| ER | Estrogen receptor |
| ER | Endoplasmic reticulum |
| ERE | Estrogen response element |
| FADD | Fas-associated protein with death domain |
| FapyAde | Formamidopyrimidine adenine derivative |
| FapyGua | Formamidopyrimidine guanine derivative |
| Fas | Fatty acid synthetase |
| FasL (Apo-1) | Fas ligand |
| FBS | Fragment-based screening |
| FdUMP | 5-Fluoro-2′-deoxyuridine-monophosphate |
| 5-FdUTP | 5-Fluoro-2′-deoxyuridine-triphosphate |
| FGAR | Formylglycinamide ribonucleotide |
| FGF | Fibroblast growth factor |
| FGFR | Fibroblast growth factor receptor |
| FGPS | Folylpolyglutamate synthetase |
| FLT-3 (CD135) | Fms-like tyrosine kinase 3 |
| FmdC | Fluoromethylenedeoxycytidine (tezacitabine) |
| 5-FP | 5-Fluoro-2-pyrimidinone |
| FPGS | Folylpolyglutamate synthetase |
| FPP | Farnesyl pyrophosphate |
| FRET | Fluorescence resonance energy transfer |
| FSH | Follicle-stimulating hormone |
| FTase | Farnesyltransferase |
| FTI | Farnesyltransferase inhibitor |
| 5-FU | 5-Fluorouracil |
| 5-FUdR | 5-Fluorouracil deoxyribonucleoside (floxuridine) |
| GAP | GTPase-activating protein |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase |

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|---|---|
| GAR | Glycinamide ribonucleotide |
| GARFT | Ribonucleotide formyltransferase |
| GBM | Glioblastoma multiforme |
| GCSF | Granulocyte colony-stimulating factor |
| GDP | Guanosine diphosphate |
| GGTase | Geranylgeranyl transferase |
| GIST | Gastrointestinal stromal tumor |
| GITR | Glucocorticoid-induced tumor necrosis factor receptor |
| GLDC | Glycine decarboxylase |
| GM-CSF | Granulocyte–macrophage colony-stimulating factor |
| GMP | Guanylic acid |
| Gn | Gonadotropin |
| GnRH (LHRH) | Gonadotropin-releasing hormone |
| Grb | Growth factor receptor bound |
| GS | γ-Secretase |
| GSI | γ-Secretase inhibitor |
| GST-π | Glutathione-*S*-transferase of the π class |
| GTP | Guanosine triphosphate |
| HDAC | Histone deacetylase |
| HDBC | Hormone-dependent breast cancer |
| HER-1 (EGFR) | Human epidermal growth factor receptor 1 |
| HER-2 (ERBB2) | Human epidermal growth factor receptor 2 |
| HGFR (c-Met) | Hepatocyte growth factor receptor |
| HGPRT | Hypoxantine guanine phosphoribosyl transferase |
| HIF | Hypoxia-inducible factor |
| HK | Hexokinase |
| HLA | Human leukocyte antigen |
| HMT | Histone methyltransferase |
| HMTA | Hexamethylenetetramine |
| HPMA | *N*-(2-hydroxypropylmethacrylamide) |
| HPV | Human papillomavirus |
| 3β-HSD/isom | 3β-Hydroxyesteroid dehydrogenase/isomerase |
| HSP | Heat shock protein |
| hTR | Human telomerase RNA component |
| HTS | High-throughput screening |
| IAPs | Inhibitors of apoptotic signals |
| IDH | Isocitrate dehydrogenase |
| IGF | Insulin-like growth factor |
| IGF-1R | Insulin-like growth factor-1 receptor |
| IκB (IKK) | Inhibitory protein of κB family |
| ImiD | Immunomodulatory drug |
| IMP | Inosinic acid |
| $IP_3$ | Inositol triphosphate |
| IPMK | Inositol polyphosphate multikinase |
| IR | Insulin receptor |
| IRS-1 | Insulin receptor substrate-1 |
| IORT | Intraoperative radiotherapy |
| IRP-1 | Iron regulatory protein 1 |
| JAK | Janus kinase |
| JNK | Jun *N*-terminal kinase |
| KLH | Keyhole limpet hemocyanin |
| KMT | Lysine methyltransferase |

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|---|---|
| LDH-A | Lactate dehydrogenase A |
| LEF | Lymphoid enhancer factor |
| LH | Luteinizing hormone |
| LH-RF | Luteinizing hormone-releasing factor |
| LRP | Lipoprotein receptor-related protein |
| LSD (KDM) | Lysine-specific demethylase |
| mAb | Monoclonal antibody |
| MAO | Monoaminooxidase |
| MAOP | Methyl 5-aminolevulinate |
| MAPK (ERK) | Mitogen-activated protein kinase (extracellular signals regulated kinase) |
| MAPKK (MEK) | MAPK kinase (MAP/ERK kinase) |
| MBD | Methyl-binding protein |
| MCL | Mantle cell lymphoma |
| Mcl-1 | Induced myeloid leukemia cell differentiation protein |
| $M_1$dG adducts | Pyrimidopurine derivatives of dG |
| MDR | Multidrug resistance |
| MDS | Myelodysplastic syndrome |
| MDSC | Myeloid-derived suppressor cell |
| MELC | Murine erythroleukemia cell |
| MetAp | Methionine aminopeptidase |
| MFR (α-FR) | Membrane folate receptor |
| MGB | Minor groove binder |
| MHC | Major histocompatibility complex |
| MKP1 | JNK-MAPK phosphatase 1 |
| MM | Multiple myeloma |
| MPE | Malignant pleural effusion |
| mRCC | Metastatic renal cell carcinoma |
| MRI | Magnetic resonance imaging |
| mRNA | Messenger RNA |
| MSC | Mesenchymal stem cell |
| MTA | Microtubule targeting agent |
| mtDNA | Mitochondrial DNA |
| MTH1 | MutT homolog 1 |
| mTOR | Mammalian target of rapamycin |
| MUTYH | Adenine/2-hydroxyadenine DNA glycosylase that excises adenine opposite 8-oxoG |
| NAE | NEDD8-activating enzyme |
| N-BP | Nitrogen-containing biphosphonate |
| NCS | Neocarzinostatin |
| NET | Neuroendocrine tumor |
| NF-κB | Nuclear transcription factor κB |
| NMC | NUT midline carcinoma |
| NPY | Neuropeptide Y |
| NQO2 | Quinone oxidoreductase 2 |
| Nrf2 | Nuclear-related factor 2 |
| NRR | Negative regulatory region |
| NSCLC | Non-small cell lung cancer |
| NTR | Nitroreductase |
| NUT | Nuclear protein in testis |
| OGG1 | 8-Oxoguanine glycosylase |
| 2-OHE2 | 2-Hydroxyestradiol |
| 8-OHGua | 8-Hydroxyguanine |
| 5′-OH-Hyd | 5′-Hydroxyhydantoin |

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|---|---|
| 5-OH-MeUra | 5-(Hydroxymethyl)uracyl |
| OPRT | Orotic phosphoribosytransferase |
| 8-oxo-dGTP | 2′-Deoxy-8-oxoguanosine triphosphate |
| OXPHOS | Oxidative phosphorylation |
| PALA | *N*-phosphonoacetyl-L-aspartate |
| PAP | Prostatic acid phosphatase |
| PBR | Phosphate-binding region |
| PCa | Prostate cancer |
| PCD | Programmed cell death |
| PDAC | Pancreatic ductal adenocarcinoma |
| PDD | Photodynamic diagnosis |
| PD-ECGF | Platelet-derived endothelial cell growth factor |
| PDEPT | Polymer-directed enzyme prodrug therapy |
| PDGF | Platelet-derived growth factor |
| PDGFR | Platelet-derived growth factor receptor |
| PDLIM2 | PDZ-LIM domain-containing protein 2 |
| PDPK1 | Phosphoinositide-dependent protein kinase-1 |
| PDT | Photodynamic therapy of cancer |
| PEG | Polyethyleneglycol |
| PEG-PGA | PEGylated glutaminase |
| PET | Positron emission tomography |
| PFK | Phosphofructokinase |
| PGA | Polyglutamic acid |
| PHGDH | Phosphoglycerate dehydrogenase |
| PI | Proteasome inhibitor |
| PI3K | Phosphatidylinositol-3-kinase |
| PIP2 | Phosphatidylinositol(4,5)-diphosphate |
| PIP3 | Phosphatidylinositol(3,4,5)-trisphosphate |
| PK | Pyruvate kinase |
| PKC | Protein kinase C |
| PLC | Phosphoplipase C |
| PLK-1 | Polo-like kinase 1 |
| PLP | Pyridoxal phosphate |
| p38 MAPK | P38 mitogen-activated protein kinase |
| PML | Promyelocite leukemia protein |
| PNA | Peptide nucleic acid |
| PNP | Purine nucleoside phosphorylase |
| PPAR | Peroxysome proliferator-activating receptor |
| PPI | Protein–protein interactions |
| PPIM | PPI modulator |
| PPP | Pentose phosphate pathway |
| PPRE | Peroxisome proliferator hormone response element |
| pRb | Retinoblastoma |
| PRL | Polypeptide hormone prolactin |
| PRLR | Polypeptide hormone prolactin receptor |
| PRPP | Phosphoribosyl pyrophosphate |
| PS | Photosensitizers |
| PTEN | Phosphatase and tensin homolog |
| PTK | Protein kinase |
| PUFA | Polyunsaturated fatty acids |
| RAP | Radiation-activated prodrug |
| RAR | Retinoic acid receptor |

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|---|---|
| RARE | Retinoic acid response element |
| RES | Reticuloendothelial system |
| RFC | Reduced folate carrier |
| RIT | Radioisotope therapy |
| RNA | Ribonucleic acid |
| RNR | Ribonucleotide reductase (equivalent to NDPR) |
| ROS | Reactive oxygen species |
| ROS1 | C-ros oncogene 1 |
| RPTK | Receptor protein kinase |
| RXR | Retinoid X receptor |
| SAC | Spindle assembly checkpoint |
| SARM | Selective androgen receptor modulator |
| SCFR (c-Kit, CD117) | Stem cell growth factor receptor |
| SERCA | Sarcoplasmic/endoplasmic reticulum calcium ATPase |
| SERM | Selective estrogen receptor modulator |
| sFRP | Secreted Frizzled-related glycoprotein |
| SGK1 | Serum glucocorticoid-regulated kinase 1 |
| SHMT | Serine hydroxymethyltransferase |
| SIRT | Silent information regulator |
| SLL | Small lymphocytic lymphoma |
| SMA | Styrene maleic acid |
| Smac | Second mitochondria-derived activator of caspase |
| Smo | Smoothended |
| SOS | Son of Sevenless |
| Sp1 | Specificity protein 1 |
| SPARC | Secreted protein acidic and rich in cysteine |
| SRIF | Somatostatin (somatotropin release-inhibiting factor) |
| SRP | Signal recognition particle |
| SST | Somatostatin |
| SSTR | Somatostatin receptor |
| STAT | Signal transducer and activator of transcription |
| STS | Steroid sulfatase |
| TACA | Tumor-associated carbohydrate antigen |
| TCF | Tumor cell factor |
| TCR | T-cell receptor |
| TDDP | *trans*-diaminedichloroplatinum(II) |
| Tdp | Tyrosyl-DNA-phosphodiesterase |
| TEM | Triethylenemelamine |
| TERT | Telomerase reverse transcriptase |
| TGF | Transforming growth factor (tumor growth factor) |
| THF | Tetrahydrofolate |
| ThyGly | Thymine glycols |
| TK | Tyrosine kinase |
| TKI | Tyrosine kinase inhibitor |
| TLR | Toll-like receptor |
| TMP | Thymidylate (thymidine monophosphate) |
| TNF | Tumor necrosis factor |
| TNFR1 | TNF receptor 1 |
| Top1 | Topoisomerase I |
| Top2 | Topoisomerase II |
| Topo | Topoisomerase |
| TP | Thymidine phosphorylase |

| | |
|---|---|
| TPMT | Thiopurine methyltransferase |
| TPZ | Tirapazamine |
| TR (TERC) | Telomerase RNA |
| TRAF2 | TNF receptor-associated factor 2 |
| TRAIL | TNF-related apoptosis-inducing ligand |
| TRAILR | TNF-related apoptosis-inducing ligand receptor |
| Treg | Regulatory T cell |
| TS | Thymidylate synthase |
| TSC | *trans*-sodium crocetinate |
| TSP1 | Trombospondin-1 |
| TSR | Thrombospondin type 1 repeat |
| TWEAK | TNF-like weak inducer of apoptosis |
| UAE (E1) | Ubiquitin-activating enzyme |
| UBC (E2) | Ubiquitin-conjugating enzyme |
| UDP | Uridine diphosphate |
| UMP | Uridine monophosphate |
| UTP | Uridine triphosphate |
| VDEPT | Virus-directed enzyme prodrug therapy |
| VDR | Vitamin D receptor |
| VEGF | Vascular endothelial growth factor |
| VEGFR | Vascular endothelial growth factor receptor |
| *VHL* gene | Von Hippel–Lindau (E3 ubiquitin protein ligase) |
| VTPT | Vascular targeted photodynamic therapy |
| WAT | White adipose tissue |
| WBRT | Whole-brain radiation therapy |
| XIAP | X-linked inhibitor of apoptosis protein |
| XMP | Xanthylic (or xantosinic) acid |

CHAPTER

# GENERAL ASPECTS OF CANCER CHEMOTHERAPY

1

CONTENTS



## 1. INTRODUCTION: SOME GENERAL COMMENTS ABOUT CANCER

*Cancer* is a collective term used to describe a group of different diseases that are characterized by the loss of control of cell growth and division, leading to a primary tumor that invades and destroys adjacent tissues. It may also spread to other regions of the body through a process known as metastasis, which is the cause of 90% of cancer deaths. Cancer remains one of the most difficult diseases to treat and is responsible for approximately 14.5% of all deaths worldwide. This incidence is increasing due to the aging of the population in most countries, including those under development. Indeed, against a widely held belief, more than two-thirds of all cancer deaths occur in low- and middle-income countries, and the estimated increase in cancer incidence by 2030, compared with 2008, will be greater in low- (82%) and lower-middle-income countries (70%) compared with the upper-middle- (58%) and high-income countries (40%).[1]

The creation in late 1971 of the U.S. National Cancer Program led by the National Cancer Institute (NCI) had as its most important consequence that the amount of basic science implied in these studies permitted the initial understanding of cancer development. Cancer has been redefined throughout the years,[2] and currently comprehensive views exist of how most cancers arise and function at the genetic and biochemical level. However, the cure of cancer continues to be a daunting objective[3] because of the high mutation potential of tumor cells and the original heterogeneity in genetic alterations of

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tumors—properties that permit the relapse of patients following initial treatment success, which creates a pressing need for alternative agents that could be used as later lines of therapy. In fact, drug resistance is still a major problem in oncology and affects old therapies, new targeted drugs, and personalized cancer treatments.

## 2 TUMORIGENESIS AND ONCOGENES: PHARMACOGENOMICS

Tumorigenesis is a multistep process whose steps reflect genetic alterations including small-scale changes in DNA sequences, such as point mutations; larger scale chromosomal aberrations, such as translocations, deletions, and amplifications; and changes that affect the chromatin structure and are associated with dysfunctional epigenetic control, such as aberrant methylation of DNA or acetylation of histones.[4] Any of these genetic alterations confers one or another type of growth advantage that drives the progressive transformation of normal cells into highly malignant cancer cells. Hanahan and Weinberg reported six hallmarks or biological capabilities acquired during the multistep development of human tumors: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.[5] Later conceptual progress added new hallmarks to this list: evading immune destruction, deregulating cellular energetics (reprogramming of energy metabolism), genomic instability and mutation, and tumor-promoted inflammation (Figure 1.1).

Furthermore, cancer is not only a cell disease but also a tissular disease in which the normal relationships between epithelial cells and their underlying stromal cells are altered.[6] In fact, tumors contain recruited cells that contribute to the acquisition of the previously mentioned hallmark traits by creating an adequate tumor microenvironment.[7]

Although cancer is not a contagious disease, infectious agents such as viruses can contribute to its origin. Most oncogenes were identified by using retroviruses, and the first evidence of the tumorigenic potential of some genes derived from studies on malignant diseases caused by them. The term *oncogene* was introduced in the mid-1960s to denote special parts of the genetic material of certain viruses

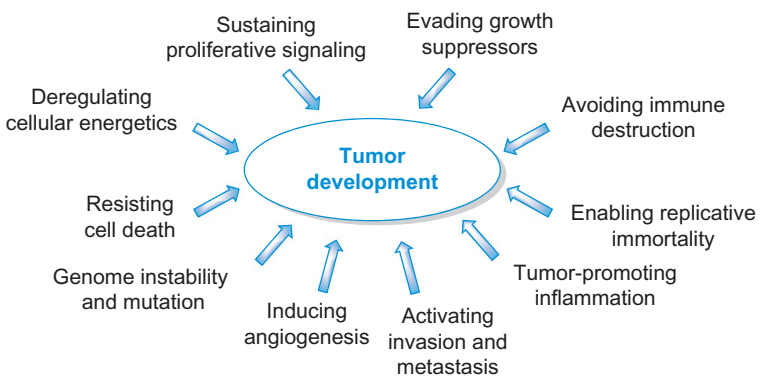



**FIGURE 1.1**

The tumorigenesis process.

that, as it was believed, could direct the transformation of a normal cell into a tumor cell. The favorite theory of the time was that virus-mediated cell-to-cell transmittance of oncogenes was the origin of all forms of cancer. This view was later proven to be incorrect because, from the standpoint of cancer development, the crucial issue is the comparison between oncogenes in normal cells (proto-oncogenes) and in tumor cells. Oncogenes are identified by the use of three-letter abbreviations; in addition, cellular and viral oncogenes are sometimes distinguished by *c-* and *v-* prefixes, respectively (e.g., *c-src* and *v-src*).

The first oncogene to be identified was *v-src*, discovered in 1970 as a component of a cancer-causing virus in chickens known as the Rous sarcoma virus. This is a member of the retroviruses family, characterized because their RNA genetic material is transcribed into DNA by the enzyme reverse transcriptase. This reverse transcription permits the integration of the genetic material of retroviruses into the chromosomal DNA in the cells. However, retroviruses play a relatively limited role in the development of cancer under natural conditions, with human T-lymphotropic virus type 1 (HTLV-1) the only known example in humans in which a retrovirus infection contributes to the origin of a cancer. Other kinds of viruses with DNA as their genetic material can also contribute to the development of tumors in humans, although other factors in addition to the virus infection are required for the cancer to develop. Certain types of papillomaviruses play a role in the development of cervical cancer in the genital tract, whereas Epstein–Barr virus is an important factor in the development of Burkitt's lymphoma in Africa and nasopharyngeal cancer in Asia.

In 1975, Bishop and Varmus demonstrated the true origin of the *v-src* oncogene by its detection in different species throughout the animal kingdom. It controls cell growth and division, and in humans it is involved in a variety of cancers, such as colon, liver, lung, breast, and pancreatic cancer.[8] Accordingly, this oncogene is not a true viral gene but, rather, a cellular gene picked up by the virus during its replication in cells.

In 1982, the first human oncogene, currently known as *H-RAS*, was cloned and characterized from the T24 bladder carcinoma.[9] Approaches to the true genetic complexity of cancer evolved as a result of the Human Genome Project (1988–2003), which led to the knowledge that among a total of approximately 25,000 human genes, mutations of approximately 200 are able to promote abnormal growth and cell division as well as evasion of programmed death, leading to cancer. Nevertheless, the regulation of growth and division of cells is much more complex than originally believed. Cellular oncogene products with different properties act in different positions in elaborate signal systems to transmit signals from one cell to another or within a single cell.

Several oncogene products function as receptors in the cytoplasmic membrane of the cells and catalyze the phosphorylation of the amino acid tyrosine. There are two groups of oncogene products with phosphokinase activity: tyrosine/phosphokinases, which lack receptor function and are located on the inside of the cytoplasmic membrane, and serine/threonine phosphokinases, which are found in the cytoplasm. Thus, oncogene products function as links in signal chains stretching from the surface of the cell to the genetic material in the nucleus. In the cytoplasm, there is one more group of oncogene products, such as Ras, that are related to the important cellular signal factors known as G proteins. Finally, several oncogene products, such as Myc, Myb, Fos, and ErbA, are located in the nucleus of the cell and direct the transcription of DNA into RNA, playing a critical role in the selection of proteins to be synthesized by the cell.

In the development of a tumor, a normal cellular oncogene may be hyperactive or an oncogene product may be altered so that it can no longer be regulated in a normal way. Oncogenes with point

mutations may cause alterations in the amino acid composition of the gene product, and they have been observed in many tumors. A well-known example of such a modification is the exchange of amino acid 12 from glycine to valine in the Ras gene product. The mutation may be more extensive, leading to the absence of part of the protein (deletion). Repeated copying of a normal oncogene can lead to its amplification in the chromosome and consequently to increased amounts of the oncogene product. The same effect can be seen when there is a reciprocal exchange of segments between chromosomes (translocation). Thus, the normal *myc* gene on chromosome 8 has been translocated to chromosome 14 in many patients with Burkitt's lymphoma, a form of non-Hodgkin's lymphoma in which cancer starts in the B cells of the immune system. Chromosome translocations occur in many different tumors.

Mutated genes that encode protein components of signal transduction pathways enable external signals such as growth and survival factors to move from the cell surface receptors to key promoter–enhancer regions along the 24 human chromosomes, where they turn up the expression of genes needed for cell growth and division and evasion of programmed cell death (apoptosis). The latter event is very important and underlies the ever-growing resistance of late-stage aggressive cancer cells to radio- and chemotherapeutic therapies. Among the multiple molecular pathways that bring about cell growth and proliferation, each with their own specific surface receptors, cytoplasmic transducers and promoters as well as enhancers of gene expression, exists much potential cross talk, which allows new DNA mutations to create new pathways to cancer when preexisting ones are blocked by a given treatment.

In 1984, Mak, a pioneer in developing genetically engineered mice known as "knockout mice" because one or more of their genes have been inactivated, demonstrated the inhibitory effect on T cells of a protein called cytotoxic T-lymphocyte antigen 4 (CTLA-4), also known as CD152 (cluster of differentiation 152). This protein is an inhibitory co-receptor that interferes with T-cell activation and proliferation.[10] This landmark discovery was an important breakthrough in understanding the human immune system, pioneering further work in the genetics of immunology that has had a direct impact on the development of personalized cancer medicine. In recent years, clinical researchers have developed techniques for re-engineering the T-cell receptor gene to target certain cancers. Such treatments, although still in the experimental stage, have yielded dramatic results in some patients, especially those with leukemia and melanoma, in part because T cells are capable of being better targeted than surgery, radiation, chemotherapy, or hormonal therapy. Those findings led to the development of ipilimumab (Yervoy®), which blocks CTLA-4 and enables T cells to proliferate and destroy certain cancer cells.[11] The editors of the journal *Science* chose cancer immunotherapy, a strategy that harnesses the body's immune system to combat tumors, as the scientific breakthrough of the year for 2013.[12]

Pharmacogenomic studies first focused on inherited genetic variants of the germline DNA, but they have been extended to somatic alterations of DNA in a tumor. These studies allow the establishment of a relationship between a drug response and the patient's genetic alterations, maximizing the chance of treatment success and minimizing the risk of toxicity. Genomic markers may be predictive, identifying whether a patient will respond or not to a drug, or prognostic, predicting the clinical course of a given cancer irrespective of treatment. Because cancer is a disease of the genome, each cancer cell may harbor many genomic alterations that differ in different tumor types, even within the same tumor in the same patient.

The impact of variations in the human genome depends on their nature and on their location. These variations may be single nucleotide polymorphisms (SNPs), variations in copy numbers, and chromosomal rearrangements (inversions and translocations). The function of proteins is altered most when nucleotide mutation alters their amino acid sequence as a consequence of nonsynonymous variations

occurring in the gene coding regions (exons). Synonymous variations, which do not alter the amino acid sequence, were thought to be silent, but they can also influence mRNA splicing, mRNA stability, and protein conformation and function.

Changes in drug response often involve germline variations that affect the pharmacokinetics of an anticancer drug by reducing the expression or activity of coded enzymes. For instance, patients with acute lymphoblastic leukemia treated with 6-mercaptopurine and who have a homozygous deficiency in thiopurine *S*-methyltransferase enzyme activity have an extreme sensitivity to this drug as a result of the accumulation of higher cellular concentrations of thioguanine nucleotides. Consequently, they have an increased risk of myelosuppression and require a substantial dose reduction.[13] However, most drug response phenotypes respond to variations in multiple genes encoding proteins that are involved in drug absorption, transport, metabolism, elimination, and mechanism of action. The aggregate effect of multiple polymorphisms or alleles that are closely linked, known as a haplotype, is frequently inherited together and, fortunately, it can be considered as a functional unit that may be represented by a marker SNP. This property allows for large sections of the genome to be studied using relatively fewer marker SNPs. Unlike germline variations, somatic mutations are not present in normal cells and are not inheritable, and they can functionally be divided into driver and passenger. Most of them are temporary and do not contribute to cancer development, but driver mutations confer growth or survival advantages in cancer cells. When they are located in oncogenes, the cancer cells become "addicted" to their function, and the oncogenes may be the target of the therapy.[14]

The success of imatinib in the treatment of chronic myeloid leukemia paved the way for the development of treatments targeting genomic aberrations in solid tumors, an approach that has been especially effective in gastrointestinal stromal tumors, breast cancer, colorectal cancer, non-small cell lung cancer, and melanoma.[15] The International Cancer Genome Consortium, the Cancer Genome Atlas, and the Cancer Genome Project have afforded comprehensive genomic information on several cancer types and have identified genomic aberrations that are potentially targetable or associated with drug resistance, thus enabling a personalized approach to cancer therapy.[16]

## 3 EARLY DIAGNOSIS OF CANCER AND ITS THERAPEUTIC RELEVANCE

The high potential for mutation of tumor cells limits the usefulness of tissue biopsy as a standard prognostic procedure for cancer because, due to the genetic diversity within a single solid tumor, cells from one end may differ from those at the other and only some mutations are shared throughout the whole mass. Accordingly, a biopsy could miss mutations that might radically change the diagnosis and prognosis of a patient, and although it can provide data about specific mutations that might make a tumor vulnerable to targeted therapies, that information may become inaccurate as the cancer evolves.[17] For an early diagnosis, prognosis, and epidemiology of cancer, it is necessary to detect specific biomarkers that, ideally, should be collected from biofluids such as blood or serum. Several genetic, epigenetic, proteomic, glycomic, and imaging biomarkers are currently used for cancer diagnosis and therapeutic monitoring, including AFP (liver cancer), Bcr-Abl (chronic myeloid leukemia), BRCA1/BRCA2 (breast/ovarian cancer), BRAF V600E (melanoma/colorectal cancer), CA-125 (ovarian cancer), CA19-9 (pancreatic cancer), CEA (colorectal cancer), EGFR (non-small cell lung carcinoma), HER-2 (breast cancer), KIT (gastrointestinal stromal tumor), PSA (prostate cancer), and S100 (melanoma).[18] Although proteins are used in the clinic to diagnose illnesses and

monitor people undergoing treatment, many of those used as cancer biomarkers are inaccurate. For example, prostate-specific antigen (PSA) can give false positives because this antigen can be elevated in blood for other reasons.

Circulating DNA (ctDNA) in human blood, first reported in the blood of cancer patients in 1977, might perform better than proteins as a biomarker because it bears mutations that are hallmarks of cancer.[19] Circulating tumor DNA is composed of genome fragments that are released when cancer cells die and float freely through the bloodstream, and it could be an excellent cancer biomarker. Unfortunately, ctDNA is not yet ready for a leading role in the clinic, mainly because the most sensitive techniques for its detection require some knowledge about which mutations to search for, and this is a laborious task that must be performed for each individual patient. One alternative is to use exome sequencing, which does not require a previous knowledge about the cancer but is prohibitively expensive. A focused approach to the therapy of lung cancer that would permit keeping costs low has been developed. This approach is based on the identification of a small fraction of the genome (0.004%) that is repeatedly mutated in these cancers. Because almost all patients with lung cancer have at least one mutation in these regions, these mutations may be found by sequencing this small fraction 10,000 times over. The method should work in almost every cancer, except in the case of brain cancers, in which the blood–brain barrier stops tumor DNA from reaching the bloodstream. Unfortunately, the potential of ctDNA as a cancer-screening tool is limited to advanced forms of cancer, which discharge relatively high levels of DNA, but it does not perform well for detecting early cancer forms.[20] It is likely that molecular characterization of a given cancer will lead to the identification of different subsets of cancer disease with a different natural history, sensitivity, and resistance to treatment. In this task, efforts to develop, validate, and implement predictive biomarkers in clinical trials and eventually in routine care are important.

Despite the current emphasis on the early diagnosis of cancer, statistical data demonstrate that advances in this field have not led to a proportional decline in later stage disease.[21] Emphasis on early diagnosis of cancer may lead to overdiagnosis—that is, the detection of tumors that if left unattended would not become clinically apparent or cause death. To minimize overdiagnosis of cancer, some oncologists have proposed a change in terminology, with the term “cancer” reserved only for lesions with a reasonable likelihood of lethal progression if left untreated.

## 4 A BRIEF HISTORY OF CANCER CHEMOTHERAPY

In addition to biological knowledge, chemistry has had varying roles in the discovery and development of anticancer drugs since the beginning of cancer therapies.[22]

Modern cancer chemotherapy has its origin in the development of nitrogen mustards as chemical weapons. Since those early years, synthetic chemistry has been extensively used to modify drug leads, especially those of natural origin, and to solve the problem of the often scarce supply of anticancer natural products by developing semisynthetic or fully synthetic strategies.

The first cytotoxic agents, most of which are still used in the clinic, were discovered through different approaches, although their mechanism was unknown. The synthesis of folate analogs was undertaken following the observation that folic acid stimulates the proliferation of acute lymphoblastic leukemia (ALL) cells, which led to the discovery of methotrexate, the first drug that induced remission in children with ALL. It is interesting to note that the development of resistance induced by old drugs such as nitrogen mustards and methotrexate was apparent since the earliest studies.

The discovery of cisplatin in the 1960s is a classic case of serendipity, when studies on the effect of an electric current on the growth of *Escherichia coli* showed that the inhibition of cell growth was not due to the electric current but, rather, to the production of a platinum complex in the electrodes. Two important anticancer drugs, doxorubicin and paclitaxel, were discovered in the screening of natural product extracts in mouse leukemia models. A more targeted approach to cancer chemotherapy was developed after the early discovery of the strong relationship between estrogens and some breast cancers.[23] The recognition that breast and prostate cancers are subject to hormonal regulation led to the introduction of antihormones that directly or indirectly target the estrogen or androgen receptors. This knowledge also led to the approval of the estrogen receptor modulator tamoxifen (Novaldex®) for cancer chemoprevention in 1998.[24,25]

Since the 1950s, the biological activities of many antitumor drug leads have been discovered through *in vitro* screening programs promoted by the NCI by using a range of cancer cell lines. In this early period, transplantable rodent tumor models characterized by a high growth rate were used for *in vivo* screening. Later, human tumor xenografts, based on transplantation of human tumor tissue into immune-tolerant animals, also became important tools for selecting antitumor drugs because these models allowed simulating a chemotherapeutic effect under conditions closer to humans. In the late 1970s and early 1980s, the role of chemotherapy was extended to preoperative and postoperative adjuvants, radiosensitizers to enhance radiation effects, and supportive therapy to increase the tolerance of the organism toward toxicity.[26] We have progressed in a few years from a lack of targets to having too many, as shown by the Cancer Gene Census, which catalogs those genes for which mutations have been causally implicated in cancer.[27] To use this information to design better drugs, improved methods for validation of these new targets are needed. In this respect, the use of high-throughput RNAi methods and genetically modified mouse models are very valuable, although removal of the target is not necessarily equivalent to its inhibition by a small molecule.

The rationale for the use of conventional cytotoxic agents as antitumor drugs was based on the notion that rapidly proliferating and dividing cells are more sensitive to these compounds that are normal cells.[28] However, as the interactions of these agents with DNA were better defined, new compounds targeting particular base sequences that may inhibit transcription factors in a more specific manner were studied. DNA was considered a molecular receptor capable of molecular recognition and triggering of response elements,[29] and the binding properties of the DNA ligands were rationalized on the basis of their structural and electronic complementarity with the functional groups present in the major and minor grooves of particular DNA sequences, which are mainly recognized by specific hydrogen bonds.[30] However, although DNA continues to be a target for anticancer chemotherapy, more recent efforts have been directed at discovering antitumor drugs specifically suited to target molecular aberrations that are specific to tumor cells.[31] This new generation of specific antitumor agents, or anticancer targeted drugs, is based on advances in molecular biology that occurred by the late 1980s, providing greatly increased understanding of regulatory and signaling networks that control fundamental cellular processes such as vascularization, cell growth and proliferation. It was then known that many of these signaling networks are enhanced in tumor cells in response to activated oncogenes.

The beginning of the twenty-first century was marked by the development of targeted therapeutics in the fight against cancer. Today, conventional chemotherapy is frequently replaced by monoclonal antibodies, kinase inhibitors, and cell differentiation or immunomodulatory agents. After the approval of trastuzumab (Herceptin®), other HER2-targeting agents, such as the small molecule lapatinib (Tykerb®) and the antibody pertuzumab (Perjeta®), were developed. Metastatic melanoma treatment

has experienced a marked revolution with the introduction of the antibody ipilimumab (Yervoy®) and the small molecule vemurafenib (Zelboraf®), which are directed against the mutated kinase bRaf V600E. The dual SRC and Abl kinase inhibitor bosutinib (Bosulif®) has improved the treatment of previously treated Philadelphia chromosome-positive chronic myeloid leukemia patients, and crizotinib (Xalkori®) is changing the management of ALK-positive lung cancers. The multikinase inhibitor regorafenib (Stivarga®) and the vascular endothelial growth factor-directed recombinant fusion protein aflibercept (Zaltrap®) are useful for metastatic colon cancer, and ruxolitinib (Jakafi®) and axitinib (Inlyta®) are used for myelofibrosis and for renal cell carcinoma, respectively. The advent of immunoconjugates in which antibodies are linked to toxins or radioisotopes has opened a new horizon for antibody-based targeted therapeutics. One example is the antibody–drug conjugate brentuximab vedotin (Adcetris®), which was approved in 2011 for the treatment of relapsed or refractory Hodgkin's lymphoma. Trastuzumab emtansine (T-DM1, Kadcyla®), approved in 2013, is another immunoconjugate for patients with metastatic breast cancer.

## 5 GENERAL COMMENTS ABOUT ANTICANCER DRUG DISCOVERY

Cancer therapy is based on local interventions such as surgery and radiotherapy, which are quite successful when viable, and on systemic chemotherapy. Approximately 50% of cancer patients are not cured by systemic chemotherapy and obtain only a prolonged survival.

Many cancer chemotherapeutic drugs currently in clinical use try to kill malignant tumor cells by inhibiting some of the mechanisms involved in cellular division. Accordingly, the antitumor compounds developed through this approach are cytostatic or cytotoxic to every dividing cell, including normal cells, and for this reason these drugs are nonspecific. However, the explosion in knowledge in tumor biology during the past decades has paved the way for specific, targeted anticancer drugs.[32] Success with the new molecularly targeted approach was demonstrated by the approval by the U.S. Food and Drug Administration (FDA) of a number of innovative drugs, both antibodies and small molecules, since the introduction of trastuzumab (Herceptin®) in 1998 as part of a treatment regimen containing doxorubicin, cyclophosphamide, and paclitaxel for the adjuvant treatment of women with node-positive, HER-2-overexpressing breast cancer.[33] Trastuzumab is a humanized monoclonal antibody that targets the extracellular region of the HER-2 receptor, leading to its internalization and degradation. The introduction in 2001 of the tyrosine kinase inhibitor imatinib (Glivec®) as a highly effective drug for patients with Philadelphia chromosome-positive chronic myeloid leukemia and gastrointestinal stromal tumors[34] was proof of the concept of effective drug development based on the knowledge of tumor biology.[35] These anticancer drugs are signal transduction inhibitors that differ from compounds developed during the cytotoxic era because they target the precise molecular mechanisms responsible for the initiation and progression of cancer. Anti-oncogene drugs have had positive results and even cured some cancers, such as lung cancers with EGFR mutations, breast cancer with mutations in HER2, or, more recently, melanoma with b-RAF mutations. Unfortunately, currently known drugs cannot replace the function of tumor suppressor genes, whose mutations are more predominant that those that activate oncogenes.

Targeted therapies may use small molecule drugs or other macromolecular structures, such as monoclonal antibodies, to bind antigens that are preferentially or exclusively present on tumor cells. Other approaches try to develop compounds that interfere with gene expression in order to suppress the

production of damaged proteins involved in carcinogenesis. In the antisense oligonucleotides approach, the mRNA translation is interfered by inhibiting the translation of the information at the ribosome, whereas in the anti-gene therapy, a direct binding to the DNA double strand inhibits transcription.[36]

The knowledge of the three-dimensional (3D) structure of these new target macromolecules, which are normally proteins, by using X-ray crystallography, permits the rational design of small molecules that mimic the stereochemical features of the macromolecule functional domains. The principal steps in structure-based drug design using X-ray techniques are summarized in Figure 1.2.

In the absence of a 3D structure of a target protein, homology criteria may be applied by using the experimental structure of similar proteins, which is especially useful in the case of individual subfamilies. The knowledge of the 3D structure of a target also permits to design and generate virtual libraries of potential drug molecules to be used for *in silico* screening.

Many targets have different subtypes and functions, which makes finding therapeutically interesting inhibitors difficult. For instance, because matrix metalloproteases (MMPs) are involved in the cleavage of some bioactive molecules besides of extracelular matrix proteins, elimination of some of them in *knockout* animals—especially MMP-3, −8, and −9—has led to the development and metastasis of tumors. For this reason, only specific MMPs must be selected as anticancer targets. An example among ligands with multiple functions is transforming growth factor-β (TGF-β). This cytokine received that name based on its ability to induce fibroblast malignancy and favor metastasis by avoiding the immune system action in the last steps of a cancer, but it has been compared to the main character in "The strange case of Dr. Jekyll and Mr. Hyde" because it may also eliminate tumors in early

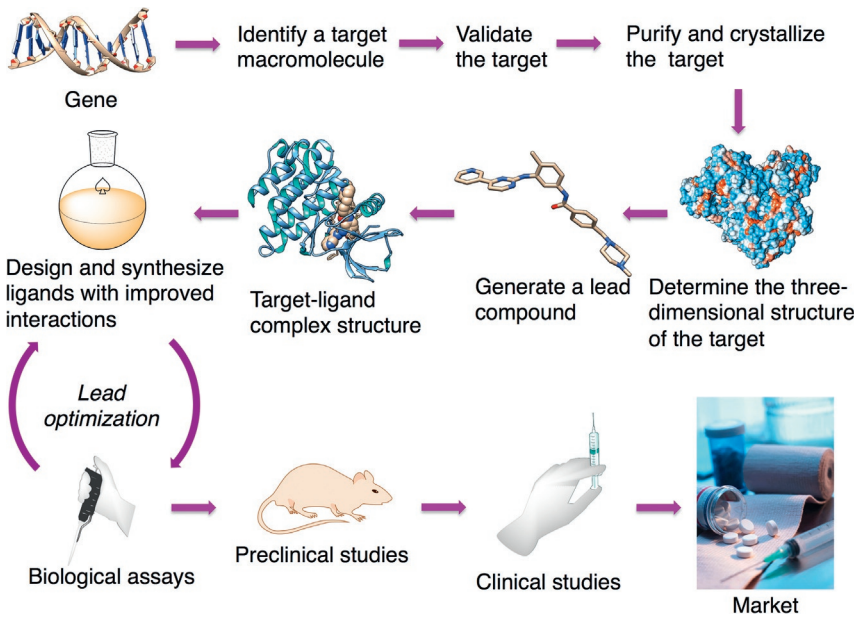



**FIGURE 1.2**

Structure-based drug design.

development. For this reason, it is necessary to select those patients for whom TGF-β inhibition is therapeutically useful.

Progress in the development of potential drug molecules is often problematic because it is difficult to convert hits into "druggable" compounds—that is, into molecules with adequate pharmaceutical properties. To this end, it is necessary to know the chemical properties of a lead compound, especially solubility and reactivity, because these properties are relevant for cellular uptake and metabolism in order to transform a lead compound into a real drug. The "druggability" of a drug candidate describes its adequate absorption, distribution, metabolism, and excretion (ADME) properties. In this task, the individualized knowledge of important metabolic enzymes, such as cytochrome P450 CYP3A4, permits improvement of the effectiveness and patient tolerance for antitumor compounds. A preliminary knowledge of ADME properties may be gained by using *in silico* techniques, although an experienced chemist can provide accurate insights into this picture by simple inspection of a given structure. The chemical properties of a drug candidate also govern its proposed formulation. In connection with ADME properties, the nonspecific biodistribution of anticancer drugs throughout the body,[37] requiring the administration of a large total dose to achieve high local concentrations in a tumor, is a major problem in cancer chemotherapy. Drug targeting aims at preferent drug accumulation in the target cells, independently of the method and route of drug administration.[38] One approach to improve the selectivity of cytotoxic compounds is the use of prodrugs that are selectively activated in tumor tissues, taking advantage of some unique aspects of tumor physiology such as selective enzyme expression, hypoxia, and low extracellular pH. More sophisticated tumor-specific delivery techniques allow the selective activation of prodrugs by exogenous enzymes (gene-directed and antibody-directed enzyme prodrug therapy) and the increased permeability of vascular endothelium in tumors (enhanced permeability and retention effect, EPR) permits that nanoparticles loaded with an antitumor drug can extravasate and accumulate inside the interstitial space, where the drug can be released as a result of normal carrier degradation.[39] In this discussion of contributions to the development of antitumor agents, it has to be mentioned that chemistry has also made possible important advances in prodrug development and in related targeted approaches, such as antibody-coupled drugs or photoactive agents.

Another major issue in cancer chemotherapy is acquired drug resistance, which is often developed by cancer cells after an initially effective treatment. Furthermore, following the development of a resistance mechanism in response to a single drug, cells can display cross-resistance to other structural and mechanistically unrelated drugs, a phenomenon known as multidrug resistance (MDR), in which ATP-dependent transporters have a significant role.[40] Resistance problems were observed during the early stages of cancer chemotherapy in the very first patient treated with a nitrogen mustard in 1942.

An additional problem in the development of anticancer drugs is the large gap from promising findings in preclinical *in vitro* and *in vivo* models to the results of clinical trials. Conventional anticancer drug screening is typically performed in the absence of accessory cells of the tumor microenvironment, and this preclinical drug testing may overestimate potential clinical activity, explaining at least in part the gap between preclinical and clinical efficacy in cancers.[41] Although a large number of clinical trials are in progress and new results are continuously being published, a statistically significant benefit is observed for very few of them.[42] In this regard, it has been claimed that to increase the efficacy of anticancer clinical trials, it is necessary to develop and use more clinically relevant cancer models. With the help of advanced engineering techniques, the development of complex 3D *in vitro* cancer

models may provide a better opportunity to understand crucial cancer mechanisms and to develop new clinical therapies.[43]

Genome-based medicine has permitted the development of personalized treatments in which effective targeted therapies may be suitable only for small subgroups of patients.[44] DNA microarray technology permits the study of alterations in the transcriptional level of entire genomes, and it may become an important tool for predicting the chemosensitivity of tumors before treatment. Pharmacogenetics, which focuses on intersubject variation in therapeutic drug effects and toxicity depending on polymorphisms, is also particularly interesting in oncology because anticancer drugs usually have a narrow margin of safety, and the parameters generally used to adjust the dose of chemotherapeutic agents (weight or body surface area) are not sufficient to overcome differences in drug disposition.[45]

Cancer stem cells (CSCs) have similar characteristics to normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer. They persist in tumors as a distinct population and cause relapse and metastasis, giving rise to new tumors. Conventional chemotherapies kill differentiated or differentiating cells that form the bulk of the tumor, but a population of CSCs can remain untouched, causing a relapse of the disease. Therefore, the development of specific therapies targeted at CSCs holds hope for improvement of survival and quality of life of cancer patients, especially for patients with metastatic disease. Cancer treatments targeting CSCs are discussed in Chapter 11, Section 7.

In parallel with these scientific developments, the cost of cancer drugs has increased exponentially. A controversial example is Provenge®, an autologous vaccine designed to stimulate the immune response to prostate cancer by targeting prostatic acid phosphatase and that costs $93,000 per treatment (2010 data). It is likely that we are witnessing a "bubble" based more on goodwill and hope than on results, and many researches think that there is an obvious need for a change of paradigm.[46]

## 6 COMBINATION THERAPY AND PERSONALIZED ANTICANCER TREATMENTS

Combination chemotherapies have been a mainstay in the treatment of disseminated malignancies for almost 60 years, but even the most successful regimens fail to cure many patients. Part of this failure is due to the absence of mechanistic information about how drugs interact to promote combination effects.[47]

It is now evident that the Ehrlich's magic bullet concept cannot be generally applied to cancer because it is a multifactorial disease and also a network problem. For this reason, the design of therapies should not focus on individual targets within a single pathway but, rather, on dysregulated cellular networks as a whole, giving place to combinatorial personalized therapies as the rational approach to overcome the failure of single drugs in complex diseases such as cancer, diabetes, and schizophrenia.[48] The strength of network biology lies in the multidimensional data that can be computationally integrated and used to identify specific and reliable therapeutic network targets to construct models of cellular decision-making processes. In this respect, in addition to protein networks, the cellular microenvironment is very important.[49]

The shift from single drug targeted therapy to combinatorial personalized therapies in cancer introduces a new challenge if we consider the whole arsenal of targeted therapies as a treatment option

for every patient. New methodologies are needed to optimize the design of combinatorial therapies to achieve the best response rates with minimal toxicity because this decision requires a transition from the one-drug/one-biomarker approach to global strategies that simultaneously assign markers to a catalog of drugs.

Classically, the drug mechanism of action refers to the description of a specific biochemical event, although in recent years, a series of drug-induced molecular/phenotypic measurements in an experimental system afford "signature"-based predictions. When these measurements arise from many drugs, the corresponding data provide multivariate signatures that fingerprint the drugs according to their relative signature similarity, but these molecular/phenotypic signatures have not been adapted to the examination of multidrug combinations. For instance, by examining the response to a diverse selection of chemotherapeutics of cells expressing short hairpin RNAs (shRNAs), which are sequences of RNA used to silence target gene expression via RNA interference (RNAi),[50] it is possible to generate a functional shRNA signature that permits the accurate grouping of these drugs into established biochemical modes of action and the prediction of mechanisms of action for molecules that are poorly characterized.[51] However, when two drugs are combined, one agent may simply reinforce the action of another or, alternatively, their combined effects may be distinct from either individual compound. Correspondingly, the shRNA signature of a combination drug would either resemble that of an individual drug or exhibit distinct genetic dependencies. In the latter case, the combined signature may be an average of individual drugs to mimic a compound not present in the combination or to adopt a novel signature. Some strategies to optimize the design of combinatorial therapies in order to achieve the best response rates with minimal toxicity have been proposed.[52]

Personalized treatments with targeted therapies designed to treat cancers carrying specific molecular alterations have been proposed as the next battle in the war against cancer. The use of clinical biomarkers to identify patients who are more likely to benefit from a given target therapy makes it possible to match a given treatment with specific patient characteristics.[53] At this point, problems associated with rapidly mutating targets and the development of drug resistances appear. For instance, in the context of breast cancer, the level of the receptor tyrosine kinase HER2/*neu* is used to select the monoclonal antibody trastuzumab (Herceptin®) as an adjuvant therapy,[54] but some patients who initially respond to the targeted therapy regress subsequently due to the occurrence of secondary molecular alterations such as the expression of the protein p95HER-2, a truncated form of the HER2 receptor lacking the extracellular domain to bind the antibody. In the context of melanoma, vemurafenib (Zelboraf®) is useful in patients with the b-Raf (V600E) mutation, where the valine-600 residue of this protein is replaced by glutamic acid.[55] However, most effectively treated patients regress after approximately 1 year due to compensatory pathways, such as receptor tyrosine kinases or N-Ras upregulation or dimerization of aberrantly spliced b-Raf (V600E).[56] In contrast to melanoma patients, colon cancer patients harboring the same b-Raf (V600E) mutation show a very limited response to this drug—a difference that can be understood by considering that treatment with vemurafenib induces feedback activation of EGFR, leading to stimulated cell growth. This adverse effect counteracts the advantages of b-Raf inhibition, especially in colon cancer, in which EGFR levels are higher than those in melamoma.[57]

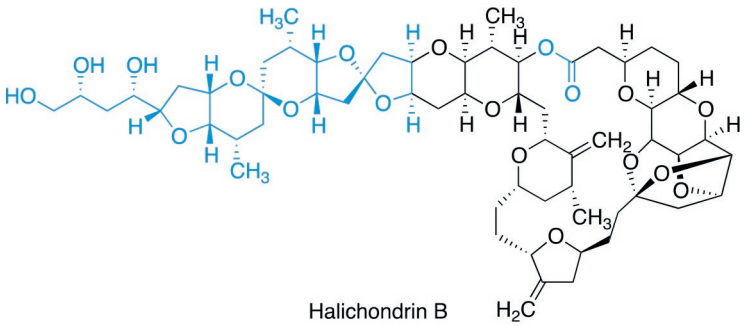
The development of monoclonal antibodies as anticancer agents has been remarkable in the past few years, but many antibody-based approaches have serious limitations because they are ineffective on target antigen-negative tumor cells, which may be preexistent in the lesion or raised through antigen shedding, masking, or therapy-induced downregulation. Other advanced strategies, such as the use of

recombinant fusion proteins in which a tumor-selective antibody fragment is fused to sTRAIL or sFasL ligands of death receptors, also fail when the targeted tumor cells are resistant to apoptosis due to one or more defects in death receptor or caspase apoptosis pathways. In these cases, to have apoptosis induction with minimal effects on normal cells, the combinatorial use of various pro-apoptotic agents working along different or complementary apoptotic signaling routes is necessary. The strategies to achieve a longer efficacy for anticancer treatments rely on the identification of specific cancer-related aberrancies in each patient, and they require the development of reliable, cost-effective, and high-throughput diagnostic tools. In this respect, laser-capture microscopy and DNA microarray technology permit the obtention of large quantities of gene expression data from individual cancer cells, although it is still difficult to extract meaningful information from these data and to connect them to tumor-specific phenomena or drug information.

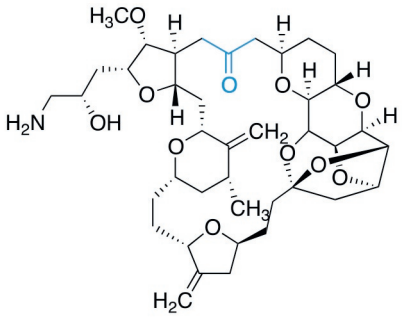
## 7 NATURAL PRODUCTS IN CANCER CHEMOTHERAPY

Since the beginning of chemotherapy, plants, microorganisms, and, more recently, marine organisms of various types have traditionally represented a main source of cytotoxic anticancer agents.[58] Nature is a source of potential chemotherapeutic agents and also of lead compounds that have provided the basis and inspiration for the semisynthesis or total synthesis of effective new drugs. The discovery of several effective anticancer agents from plants may be attributed, directly or indirectly, to a history of use of the relevant plant in traditional medicine. From the mechanistic standpoint, microtubules are a very frequent target of cytotoxic natural products.

A large number of drugs in clinical use as anticancer drugs are of natural product origin, and it has been estimated that approximately 80% of new chemical entities with small-molecule structures introduced during the period from 1950 to 2010 in this field were natural products or were natural-product inspired (small molecules, in turn, represent 77% of the total).[59] Despite this statistic, pharmaceutical companies have neglected the development of potential natural drug candidates. The main reason for this reluctance lies primarily in supply problems, which make necessary the development of synthetic routes often long and difficult to scale up because of the structural complexity of natural products. It is becoming increasingly apparent, however, that the unguided production of vast libraries of compounds is unlikely to result in the identification of new drugs, whereas natural products have in general several functional groups that are located in a precise 3D position, providing specific interactions with target molecules. It is often assumed that secondary metabolites have been optimized through evolution and that, consequently, they may be considered as highly advanced lead compounds in which further optimization of activity is difficult.[60] Nevertheless, in many cases, some parts of the complex structure of a natural product act only as a framework to position determined atoms, and simpler analogs may be developed without considerable loss of activity. For this reason, structural modification of natural products is often directed to find a possible simplest portion that maintains most of the biological activity—that is, its pharmacophoric unit. One example of this approach is the discovery of the antitumor agent eribulin (E-7389) in the development of synthetic strategies to obtain halichondrin B. Studies revealed that deletion of a large portion of this natural product and the replacement of the unstable lactone by a ketone function did not significantly affect its antimitotic properties (see Chapter 9, Section 2.1.2).[61] Eribulin was approved to treat several cancers and is under clinical trials for other types.



Halichondrin B



Eribulin

Although combinatorial chemistry, diversity-oriented synthesis, and high-throughput screening (HTS) of large compound libraries are important technologies in the discovery of bioactive molecules, the role of natural sources in providing new cytotoxics continues to be relevant.[62,63] Indeed, the notion that the use of natural-product templates combined with chemical modifications leading to more selective analogs has a better chance of success than combinatorial approaches is gaining acceptance. In other words, it appears that, at least in the anticancer field, "nature has already carried out the combinatorial chemistry" and all we have to do is refine the structures.[64] These ideas have led to an increased interest in natural products as drug candidates.[65]

## 8 A BRIEF COMMENT ABOUT CANCER NANOTECHNOLOGY

Nanotechnology is a field of applied science that covers a broad range of topics in which matter is controlled on a scale of 1–100 nm. Its application to cancer chemotherapy includes the use of nanovectors for the targeted delivery of antitumor compounds and imaging contrast agents, aiming at increasing the efficacy per dose of therapeutic or imaging contrast formulations.[66]

Liposomes, which are the simplest forms of nanovectors, use the EPR effect to increase drug concentration at tumor sites, and they were first applied to anthracyclines in order to avoid their cardiotoxicity. The refinement of liposomes and their application in cancer chemotherapy is still an active field of research, although other novel drug-delivery modalities have appeared.[67,68] In general, a nanovector has a core constituent material, a therapeutic and/or imaging payload, and biological surface

modifiers to enhance biodistribution and tumor targeting. Among several types of nanoparticles directed to enhance the properties of magnetic resonance imaging (MRI) contrast agents,[69] dendrimers, which are self-assembling polymers, have been used in mouse models of breast cancer to study the lymphatic drainage by MRI.[70]

Beyond nanovectors, several nanotechnologies are realistic candidates for the precise patterning of biological molecules, including DNA microarrays and surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectroscopy.[71]

Microarrays are devices used for molecular diagnostics, genotyping, and biomarking. They are single-stranded DNA probes that are prepared through a sequential procedure that implies selective ultraviolet deprotection of hydroxyl groups. With the ability to control the molecular depositions of polynucleotides in a nanometer range, the information density might be packed in nanoarrays directed at nucleic acids[72] or at the detection of proteomic profiles.[73]

## 9 SUMMARY OF FDA-APPROVED ANTICANCER DRUGS

Tables 1.1–1.9 summarize the main drugs approved by the FDA for use as anticancer agents according to the chapter in which they first appear.[74] The preponderance in recent years of targeted approaches to cancer treatment over cytotoxicity-based chemotherapy is readily appreciated. Orphan drug designations, drug combinations, and adjuvants in cancer therapy have been excluded.

**Table 1.1 FDA-Approved Anticancer Drugs Described in Chapter 2**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| 6-Mercaptopurine (Purinethol®) | Purine biosynthesis inhibitor | 1953 |
| Methotrexate | Dihydrofolate reductase inhibition | 1962 |
| 5-Fluorouracil (5-FU) | Thymidylate synthase inhibitor | 1966 |
| Tegafur (Ftorafur®) | Thymidylate synthase inhibitor | 1967 |
| Hydroxyurea (Hydrea®) | Ribonucleotide reductase inhibitor | 1967 |
| Cytarabine (Ara-C, Cytosar U®) | DNA synthesis inhibitor | 1969 |
| Floxuridine (FUDR) | Thymidylate synthase inhibitor | 1970 |
| L-Asparaginase (Elspar®) | Hydrolysis of circulating L-asparagine | 1978 |
| Pentostatin (Nipent®) | Adenosine deaminase inhibitor | 1991 |
| Fludarabine (Fludara®) | DNA synthesis inhibitor | 1991 |
| Cladribine (Litak®) | DNA synthesis inhibitor | 1992 |
| Trimetrexate (Neutrexin®) | DHFR inhibitor | 1994 |
| Gemcitabine (Gemzar®) | DNA synthesis inhibitor | 1996 |
| Capecitabine (Xeloda®) | DNA synthesis inhibitor | 1998 |
| Raltitrexed (Tomudex®) | Thymidilate synthase inhibitor | 1998 |
| Azacitidine (Vidaza®) | DNA synthesis inhibitor | 2004 |
| Clofarabine (Clolar®) | DNA synthesis inhibitor | 2004 |
| Pemetrexed (Alimta®) | Thymidilate synthase and dihydrofolate reductase inhibitor | 2004 |

Continued

**Table 1.1 FDA-Approved Anticancer Drugs Described in Chapter 2—cont'd**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| Eniluracil | DNA synthesis inhibitor | 2005 |
| Nelarabine (Arranon®) | DNA synthesis inhibitor | 2005 |
| Pegaspargase (Oncaspar®) | Hydrolysis of circulating L-asparagine | 2006 |
| Fludarabine (Fludara®) | DNA synthesis inhibitor | 2008 |
| Pralatrexate (Folotyn®) | Dihydrofolate reductase inhibitor | 2009 |
| *Erwinia chrysanthemi* asparaginase (Erwinaze®) | Hydrolysis of circulating L-asparagine | 2011 |

**Table 1.2 FDA-Approved Anticancer Drugs Described in Chapter 3**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| Medroxyprogesterone (Provera®) | Gestagen receptor agonist | 1959 |
| Testolactone (Teslac®) | Steroidal aromatase inhibitor | 1970 |
| Mitotane (Lysodren®) | Glucocorticoid biosynthesis inhibitor | 1970 |
| Megestrol acetate (Megace®) | Gestagen receptor agonist | 1971 |
| Tamoxifen (Nolvadex®) | Antiestrogen | 1977 |
| Aminoglutethimide (Cytadren®) | Aromatase inhibitor | 1981 |
| Leuprorelin (Lupron®) | GnRH (LHRH) agonist | 1985 |
| Lupron Depot® | | 1996 |
| Flutamide (Eulexin®, Drogenil®) | Antiandrogen | 1989 |
| Goserelin (Zoladex®) | GnRH (LHRH) agonist | 1989 |
| Finasteride (Proscar®) | 5α-Reductase inhibitor | 1992 |
| Bicalutamide (Casodex®) | Antiandrogen | 1995 |
| Anastrozole (Arimidex®) | Aromatase inhibitor | 1995 |
| Goserelin acetate (Zoladex®) | GnRH (LHRH) agonist | 1996 |
| Nilutamide (Nilandron®) | Antiandrogen | 1996 |
| Letrozole (Femara®) | Aromatase inhibitor | 1997 |
| Octeotride (Sandostatin®) | Somatostatin analog | 1998 |
| Exemestane (Aromasin®) | Aromatase inhibitor | 1999 |
| Alitretinoin (Panretin®) | Retinoid | 1999 |
| Bexarotene (Targretin®) | Retinoid | 1999 |
| Triptorelin (Trelstar®) | GnRH (LHRH) agonist | 2000 |
| Fulvestrant (Faslodex®) | Antiestrogen | 2002 |
| Dutasteride (Avodart®) | 5α-Reductase inhibitor | 2002 |
| Abarelix (Plenaxis®) | GnRH (LHRH) antagonist | 2003 |
| Histrelin (Vantas®) | GnRH (LHRH) agonist | 2004 |
| Raloxifene (Evista®) | Antiestrogen | 2007 |
| Degarelix (Firmagon®) | GnRH (LHRH) antagonist | 2008 |
| Abiraterone (Zytiga®) | CYP17A1 inhibitor | 2011 |
| Enzalutamide (Xtandi®) | Antiandrogen | 2012 |
| Pasireotide (Signifor®) | Somatostatin analog | 2012 |

**Table 1.3 FDA-Approved Anticancer Drugs Described in Chapter 4**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| Actinomycin D (Cosmege®) | ROS generation | 1964 |
| Bleomycin (Blenoxane®) | ROS generation | 1973 |
| Doxorubicin (Adriamycin®) | ROS generation | 1974 |
| Daunomycin (Cerubidine®) | ROS generation | 1979 |
| Idarubicin (Idamycin®) | ROS generation | 1990 |
| Valrubicin (Valstar®) | ROS generation | 1998 |
| Porfimer sodium oligomer (Photophrin®) | Photodynamic therapy of cancer | 1995 |
| Epirubicin (Ellence®) | ROS generation | 1999 |
| Methoxsalen (Uvadex®) | Non-porphirin photosensitizers | 1999 |
| $^{90}$Y-ibritumomab tiuxetan (Zevalin®) | Radiolabeled monoclonal antibody | 2002 |
| $^{111}$In-capromab pendetide (ProstaScintas®) | Radiolabeled monoclonal antibody | 2002 |
| $^{131}$I-tositumomab (Bexxar®) | Radiolabeled monoclonal antibody | 2003 |
| ALA (Levulan®) | Photosensitizer | 2004 |
| Amifostine (Ethiol®) | Radioprotector | 2008 |
| Alpharadin (Xofigo®, $^{233}RaCl_2$) | Radiopharmaceutical | 2013 |

**Table 1.4 FDA-Approved Anticancer Drugs Described in Chapters 5 and 6**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| Mechlorethamine (Mustargen®) | DNA alkylation | 1949 |
| Triethylenemelamine (Tetramine®) | DNA alkylation | 1953 |
| Busulfan (Myleran®) | DNA alkylation | 1954 |
| Chlorambucil (Leukeran®) | DNA alkylation | 1957 |
| Cyclophosphamide (Cytoxan®) | DNA alkylation | 1959 |
| Thiotepa (Thioplex®) | DNA alkylation | 1959 |
| Melphalan (L-PAM, Alkeran®) | DNA alkylation | 1959 |
| Uracil mustard | DNA alkylation | 1962 |
| Pipobroman (Vercyte®) | Unknown | 1966 |
| Procarbazine (Matulane®) | DNA alkylation | 1969 |
| Mitomycin C (Mutamycin®) | Minor groove alkylation | 1974 |
| Dacarbazine (DTIC-Dome®) | DNA alkylation | 1975 |
| Lomustine (CCNU, CeeNU®) | DNA alkylation | 1976 |
| Carmustine (BiCNU®) | DNA alkylation | 1977 |
| Cisplatin (Platinol®) | DNA complexation | 1978 |
| Streptozotocin (Zanosar®) | DNA alkylation | 1982 |
| Ifosfamide (Mitoxana®) | DNA alkylation | 1988 |
| Carboplatin (Paraplatin®) | DNA complexation | 1989 |
| Altretamine (Hexalen®) | DNA alkylation | 1990 |
| Melphalan (Alkeran®) | DNA alkylation | 1993 |
| Busulfan (Myleran®) | DNA alkylation | 1999 |
| Temozolomide (Temodal®, Temodar®) | DNA alkylation | 1999 |
| Oxaliplatin (Eloxatin®) | DNA complexation | 2002 |
| Bendamustine (Ribomustin®) | DNA alkylation | 2008 |

**Table 1.5 FDA-Approved Anticancer Drugs Described in Chapters 7 and 8**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| Actinomycin D (Cosmege®) | DNA intercalation | 1964 |
| Doxorubicin (DOX, Adriamycin®) | Topoisomerase II inhibition | 1974 |
| Daunomycin (DNR, Cerubidine®) | Topoisomerase II inhibition | 1979 |
| Etoposide (VP-16-213) | Topoisomerase II inhibition | 1983 |
| Mitoxantrone (Novantrone®) | Topoisomerase II inhibition | 1988 |
| Idarubicin (Idamycin®) | Topoisomerase II inhibition | 1990 |
| Teniposide (Vumon®) | Topoisomerase II inhibition | 1992 |
| Topotecan (Hycamtin®) | Topoisomerase I inhibition | 1996 |
| Irinotecan (Camptosar®) | Topoisomerase I inhibition | 1996 |
| Valrubicin (Valstar®) | Topoisomerase II inhibition | 1998 |
| Epirubicin (Ellence®) | Topoisomerase II inhibition | 1999 |
| Mithramycin A (Mithracin®) | Histone methyltransferase inhibitor | 1970 |
| 5-Azacitidine (Vidaza®) | DNA methyltransferase inhibitor | 2004 |
| Decitabine (Dacogen®) | DNA methyltransferase inhibitor | 2006 |
| Vorinostat (SAHA, Zolinza®) | Histone deacetylase inhibitor | 2006 |
| Romidepsin (FK-228, Istodax®) | Histone deacetylase inhibitor | 2009 |
| Belinostat (PDX-101, Beleodaq®) | Histone deacetylase inhibitor | 2014 |

**Table 1.6 FDA-Approved Anticancer Drugs Described in Chapter 9**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| Vincristine (Oncovin®) | Microtubule polymerization inhibitor | 1963 |
| Vinblastine (Velban®) | Microtubule polymerization inhibitor | 1965 |
| Estramustine (Estracyt®, Emcyt®) | Microtubule polymerization inhibitor | 1981 |
| Paclitaxel (Taxol®) | Microtubule-stabilizing agent | 1992 |
| Vinorelbine tartrate (Navelbine®) | Microtubule polymerization inhibitor | 1994 |
| Docetaxel (Taxotere®) | Microtubule-stabilizing agent | 1996 |
| Ixabepilone (Ixempra®) | Microtubule-stabilizing agent | 2007 |
| Eribulin mesylate (Halaven®) | Microtubule polymerization inhibitor | 2010 |
| Cabazitaxel (Jevtana®) | Microtubule-stabilizing agent | 2010 |

**Table 1.7 FDA-Approved Anticancer Drugs Described in Chapters 10 and 11**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| Trastuzumab (Herceptin®) | HER-2 inhibitor | 1998 |
| Arsenic trioxide (Trisenox®) | Apoptosis induction | 2000 |
| Imatinib mesylate (Glivec®) | Bcr-Abl inhibitor | 2001 |

**Table 1.7 FDA-Approved Anticancer Drugs Described in Chapters 10 and 11—cont'd**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| Gefitinib (Iressa®) | EGFR inhibitor | 2003 |
| Erlotinib (Tarceva®) | EGFR inhibitor | 2004 |
| Cetuximab (IMC-C225, Erbitux®) | EGFR inhibitor | 2004 |
| Bevacizumab (Avastin®) | VEGF inhibitor | 2004 |
| Sorafenib (Nexavar®) | Multikinase inhibitor | 2005 |
| Dasatinib (Sprycel®) | Bcr-Abl and Src inhibitor | 2006 |
| Sunitinib (Sutent®) | VEGFR inhibitor | 2006 |
| Panitumumab (Vectibix®) | EGFR inhibitor | 2006 |
| Nilotinib (Tasigna®) | Bcr-Abl inhibitor | 2007 |
| Temsirolimus (Torisel®) | mTOR inhibitor | 2007 |
| Lapatinib (Tyverb®) | EGFR and HER-2 inhibitor | 2007 |
| Plerixafor (Mozobil®) | CXCR4 inhibition | 2008 |
| Pazopanib (Votrient®) | VEGFR inhibitor | 2009 |
| Everolimus (Afinitor®) | mTOR inhibitor | 2009 |
| Vemurafenib (Zelboraf®) | Raf inhibitor | 2011 |
| Crizotinib (PF-02341066, Xalkori®) | ALK and c-Met inhibitor | 2011 |
| Vandetanib (Caprelsa®) | VEGFR and EGFR inhibitor | 2011 |
| Ruxolitinib (Jakavi®) | JAK inhibitor | 2011 |
| Bosutinib (SKI-606, Bosulif®) | Bcr-Abl and Src inhibitor | 2012 |
| Cabozantinib (Cometriq®) | Inhibitor of VEGFR and related receptors | 2012 |
| Ponatinib (AP24534, Iclusig®) | Bcr-Abl inhibitor | 2012 |
| Regorafenib (Stivarga®) | Raf inhibitor | 2012 |
| Omacetaxine mepesuccinate (Synribo®) | Inhibitor of protein synthesis | 2012 |
| Pertuzumab (2C4, Perjeta®) | HER-2 inhibitor | 2012 |
| Axitinib (Inlyta®) | Inhibitor of VEGFR and related receptors | 2012 |
| Dabrafenib (Tafinlar®) | Raf inhibitor | 2013 |
| Trametinib (Mekinist®) | MEK inhibitor | 2013 |
| Afatinib (Gilotrif®) | EGFR inhibitor | 2013 |
| Ibrutinib (PCI-32765, Imbruvica®) | Bruton's tyrosine kinase inhibitor | 2013 |
| Idelalisib (Zydelig®) | PI3K inhibitor | 2014 |
| Ceritinib (Zykadia®) | ALK inhibitor | 2014 |
| Bortezomib (Velcade®) | Proteasome inhibitor | 2003 |
| Thalidomide (Thalomid®) | Angiogenesis inhibition | 2006 |
| Lenalidomide (Revlimid®) | Angiogenesis inhibition | 2006 |
| Carfilzomib (Krypolis®) | Proteasome inhibitor | 2012 |
| Vismodegib (GDC-0449, Erivedge®) | Smo receptor inhibitor | 2012 |
| Pomalidomide (CC- 4047, Imnovid®) | Angiogenesis inhibition | 2013 |
| Lenvatinib (Lenvima®) | VEGFR-2 inhibitor | 2015 |

**Table 1.8 FDA-Approved Anticancer Drugs Described in Chapter 12**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| Aldesleukin (Proleukin®) | Modified human IL-2 | 1992 |
| Rituximab (Rituxan®) | Anti-CD20 | 1997 |
| Recombinant interferon α-2b (Intron A®) | Immunotherapeutic agent | 1997 |
| Denileukin diftitox (Ontak®) | IL-2 receptor antagonist | 1999 |
| Alemtuzumab (Campath®) | Anti-CD-52 | 2001 |
| $^{131}$I-tositumomab (Bexxar®) | Anti-CD20 with radioactive iodine | 2003 |
| Imiquimod (Aldara®, Zyclara®) | Agonist of toll-like receptors 7 and 8 | 2004 |
| Gardasil® | Cancer vaccine | 2006 |
| Plerixafor (Mozobil®) | Chemokine receptor-4 (CXCR4) antagonist | 2008 |
| Cervarix® | Human papillomavirus vaccine | 2009 |
| Ofatumumab (Arzerra®) | Anti-CD20 | 2009 |
| Sipuleucel-T (Provenge®) | Autologous vaccine | 2010 |
| Denosumab (Prolia®) | Anti-RankL | 2010 |
| Brentuximab vedotin | Anti-CD30 | 2011 |
| Ipilimumab (Yervoy®) | Anti-CTLA-4 | 2011 |
| Catumaxomab (Removab®) | Anti-EpCAM, CD3, and FcγRs | 2011 |
| Ziv-aflibercept (Zaltrap®) | VEGF inhibitor | 2011 |
| Obinutuzumab (Gazyva®) | Anti-CD20 | 2013 |
| Ramucirumab (Cyramza®) | VEGFR-2 | 2014 |
| Pembrolizumab (Keytruda®) | PD-1 receptor antibody | 2014 |

**Table 1.9 FDA-Approved Anticancer Drugs Described in Chapters 13–15**

| Drug | Mechanism of Action | Approval Date (First Indication) |
|---|---|---|
| Pegaspargase (Oncaspar®) | Hydrolysis of circulating L-asparagine | 1994 |
| Liposomal doxorubicin (Doxil®) | ROS generation, topoisomerase II inhibition | 1995 |
| Liposomal daunorubicin (DaunoXome®) | ROS generation, topoisomerase II inhibition | 1996 |
| Liposomal cytarabine (Depocyt®) | Inhibition of DNA strand elongation | 1999 |
| Gemtuzumab ozogamicin (Mylotarg®) | Radical-induced DNA strand scission | 2000 |
| Pegfilgrastim (Neulasta®) | Granulocyte colony-stimulating factor (G-CSF) | 2002 |
| Albumin-bound paclitaxel (Abraxane®) | Microtubule stabilization | 2005 |
| Ibritumomab tiuxetan ($^{90}$Y) (Zevalin®) | Ionizing radiation | 2009 |
| Genexol-PM® | Microtubule stabilization | 2010 |
| Brentuximab vedotin (Adcetris®) | (Antibody–drug conjugate) | 2011 |
| Marqibo® | (Liposomal vincristine) | 2012 |
| Oxorubicin liposome (LipoDox®) | (Liposomal doxorubicin) | 2013 |
| Trastuzumab-DM1, ado-trastuzumab | Microtubule depolymerization | 2013 |
| Ado-trastuzumab emtansine (Kadcyla®) | Microtubule depolymerization | 2013 |
| Tamoxifen (Nolvadex®, Valodex®) | Selective estrogen receptor modulator (SERM) | 1998 |

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CHAPTER

# ANTIMETABOLITES THAT INTERFERE WITH NUCLEIC ACID BIOSYNTHESIS

2

## CONTENTS



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## 1 INTRODUCTION

Antimetabolites can be defined as analogs of naturally occurring compounds that interfere with their formation or utilization, thus inhibiting essential metabolic routes. Although the enzymes inhibited by antimetabolites are also present in normal cells, some selectivity toward cancer cells is possible due to their faster division rates.

Most antimetabolites interfere with nucleic acid synthesis, and for this reason we study in this chapter the antitumor compounds that hamper the production of DNA or RNA by a variety of mechanisms, including the following:

1. Competition for binding sites of enzymes that participate in essential biosynthetic processes
2. Incorporation into nucleic acids, which inhibits their normal function and triggers the apoptosis process

Because of this mode of action, most antimetabolites have high cell cycle specificity.

A brief outline of DNA biosynthesis is given in Figure 2.1, including the main steps where antimetabolite drugs discussed in this chapter exert their action.

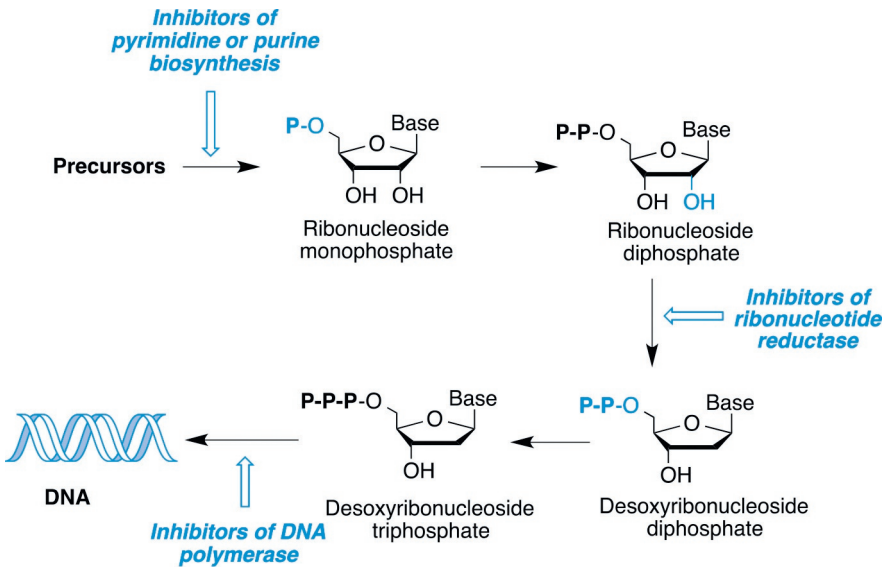



**FIGURE 2.1**

Types of anticancer drugs that interfere with DNA biosynthesis.

Although clinically useful antimetabolites ultimately inhibit DNA (and sometimes RNA) synthesis, their site of action may be separated many steps away from these reactions. Specific interference with the *de novo* nucleic acid pathways in cancer cells is probably not possible because tumoral and normal cells use the same biosynthetic routes. Nevertheless, some antimetabolites are remarkably effective against some human cancers and are still one of the bases of cancer chemotherapy.

## 2 INHIBITORS OF THE BIOSYNTHESIS OF URIDYLIC ACID

The biosynthesis of pyrimidine nucleotides starts with the construction of the heterocyclic system by carbamoylation of aspartate followed by cyclization to dihydroorotate. Its dehydrogenation gives orotate, which then reacts with phosphoribosyl pyrophosphate (PRPP) to give orotidylate. Finally, uridylic acid (uridine monophosphate (UMP)) is generated by decarboxylation (Figure 2.2). UMP is the precursor to other pyrimidine nucleotides, after its conversion to the corresponding nucleoside triphosphate (UTP).

Among the many compounds known to inhibit reactions of this pathway, we mention only *N*-phosphonoacetyl-L-aspartate (PALA), an inhibitor of aspartate transcarbamoylase that acts as a

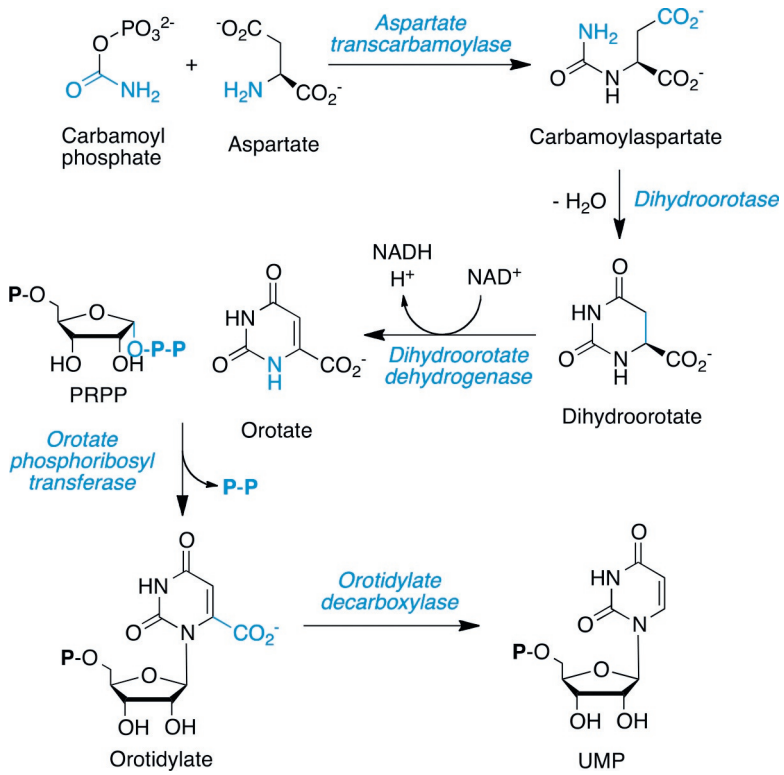



**FIGURE 2.2**

Biosynthesis of pyrimidine nucleotides.

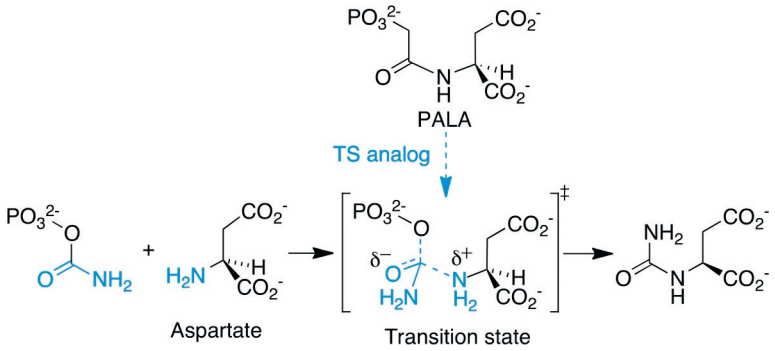



**FIGURE 2.3**

Inhibition of aspartate transcarbamoylase by PALA.

transition state analog (Figure 2.3). This compound has undergone some clinical trials, normally in combination with 5-fluorouracil, another pyrimidine antimetabolite.[1]

## 3 INHIBITORS OF RIBONUCLEOTIDE REDUCTASE

The biosynthesis of 2′-deoxyribonucleotides, the immediate precursors of DNA, involves the replacement of the 2′-OH group by a hydrogen atom (Figure 2.4). This reaction takes place on ribonucleoside-5′-diphosphates and is catalyzed by the enzyme ribonucleotide reductase (RNR), also known as nucleoside diphosphate reductase (NDPR). RNR is the rate-determining enzyme in the supply of deoxynucleotides, and its substrates are ADP, GDP, CDP, and UDP. Deoxythymidine diphosphate (dTDP) is synthesized by another enzyme (thymidylate kinase) from deoxythymidine monophosphate (dTMP). Ribonucleotide reductase thus plays a central role in cell growth and proliferation by ensuring a balanced supply of nucleotide precursors for DNA synthesis, and it has been identified as an important target for hematologic malignancies.[2]

### 3.1 STRUCTURE AND CATALYTIC CYCLE OF RIBONUCLEOTIDE REDUCTASE

The most extensively studied ribonucleotide reductase is that from *Escherichia coli*, which is considered as a suitable prototype for the mammalian enzyme. In eukaryotes, ribonucleotide reductase has two subunits, with each containing a dinuclear iron center that generates an essential stable tyrosyl

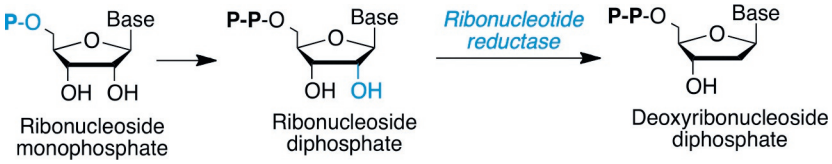



**FIGURE 2.4**

Biosynthesis of 2-deoxyribonucleotides.

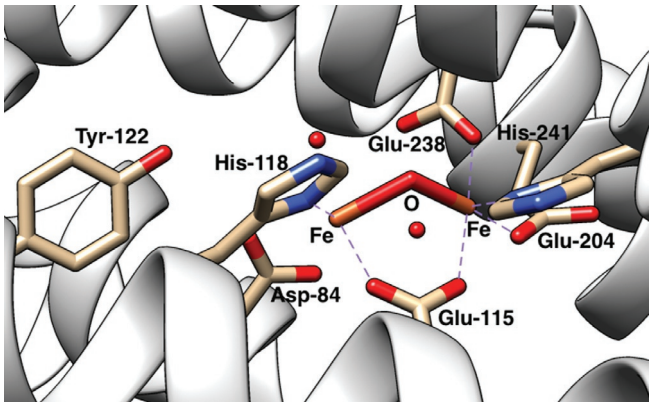



**FIGURE 2.5**

Structure of the iron binding site in the ribonucleotide reductase R2 subunit from *E. coli*, generated from Protein Data Bank reference 1AV8 and displayed with Chimera 1.8.1.

radical by one electron oxidation of a nearby tyrosine (Tyr-122) deeply buried inside the protein, in a highly hydrophobic environment. The Fe cations are coordinated to a number of residues (Asp-84, His-118, His-241, Glu-115, Glu-204, and Glu-238) and two molecules of water (Figure 2.5).

The enzyme generates and stabilizes a tyrosyl radical through a redox process that transforms the initial Fe(II) complex into a binuclear oxo-bridged Fe(III) complex. A high-resolution X-ray diffraction study has shown that the first iron atom is pentacoordinate, although it maintains an octahedral structure, and the second one is hexacoordinate (Figure 2.6).[3]

Although the Tyr-122 radical triggers the reductive process, it is too far away from the catalytic site. Therefore, it must generate a second radical in the vicinity of the substrate, probably a thiyl radical from Cys-439. The cysteine radical then abstracts the $C_{3'}$–H atom of the nucleoside diphosphate substrate and generates the anion-radical **2.1**, with prior or simultaneous deprotonation of the $C_{3'}$–OH group by the Glu-441 residue of the enzyme. Two cysteine residues, probably Cys-225 and Cys-462, form the redox-active sulfhydryl pair responsible for the reduction of this radical. Thus, protonation of the $C_{2'}$–OH and subsequent elimination of a molecule of water yields a cation that is stabilized by migration of the unpaired electron from C-3′ to C-2′ to give **2.2**. The Cys-462 mercapto group transfers a proton and one electron to this radical to give **2.3**, with concomitant formation of a disulfide anion radical, which then transfers one electron to the carbonyl group in **2.3**, leading to **2.4**. Radical **2.4** is transformed into **2.5** by a mechanism reverse to the one that produced **2.1**, and the active center of the enzyme is finally regenerated by reduction of the newly formed disulfide unit by thioredoxin, an ubiquitous protein that has a pair of proximal cysteine residues, which reacts with the oxidized form of ribonucleotide reductase via disulfide exchange (Figure 2.7).[4]

It is interesting to note that the enzymatic reaction of ribonucleotide reductase is initiated by the formation of a radical (species **2.2**), even though the reactions leading to reductive elimination of the $C_{2'}$–OH group are ionic. The reason for this type of mechanism may be the stabilization of **2.2** through the effect of the radical at C-3 on the intermediate carbocation formed at C-2, as shown by the following resonance structures:

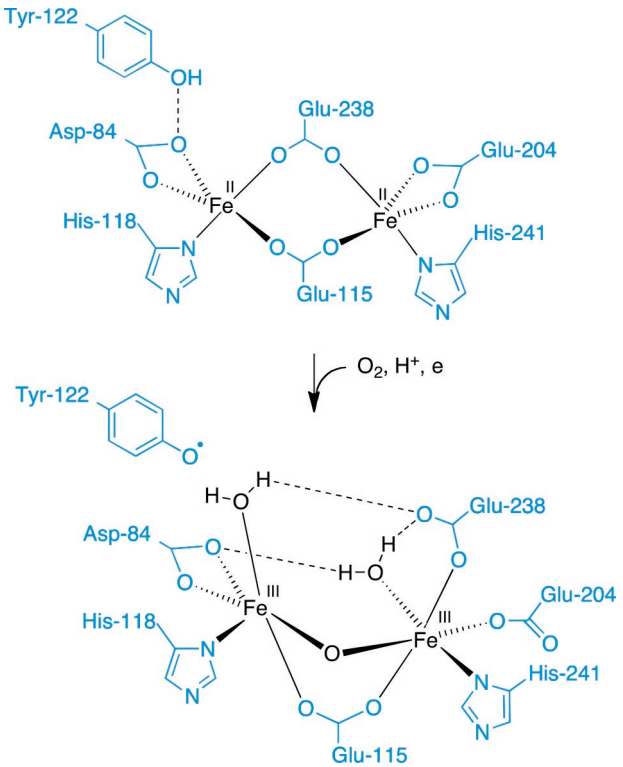
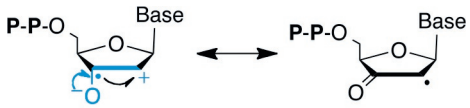



**FIGURE 2.6**

Generation of a tyrosyl radical in the active site of ribonucleotide reductase.




**2.2**

## 3.2 GALLIUM SALTS AND COMPLEXES

Gallium ions can inhibit DNA synthesis through substitution of $Ga^{3+}$ for $Fe^{3+}$ in the $M_2$ subunit of ribonucleotide reductase. Furthermore, their synergy with hydroxyurea has been demonstrated.[5] $Ga^{3+}$ is usually administered as its nitrate salt or as gallium maltolate, a complex formed by a $Ga^{3+}$ cation coordinated and three maltolate ligands, derived from 2-methyl-3-hydroxy-4-pyrone (maltol). Clinical studies have shown gallium nitrate to have significant antitumor activity against non-Hodgkin's lymphoma and bladder cancer,[6] but only 60% of patients show a positive response due to resistance problems associated with decreased Ga uptake and other mechanisms. It shows renal toxicity because it tends to

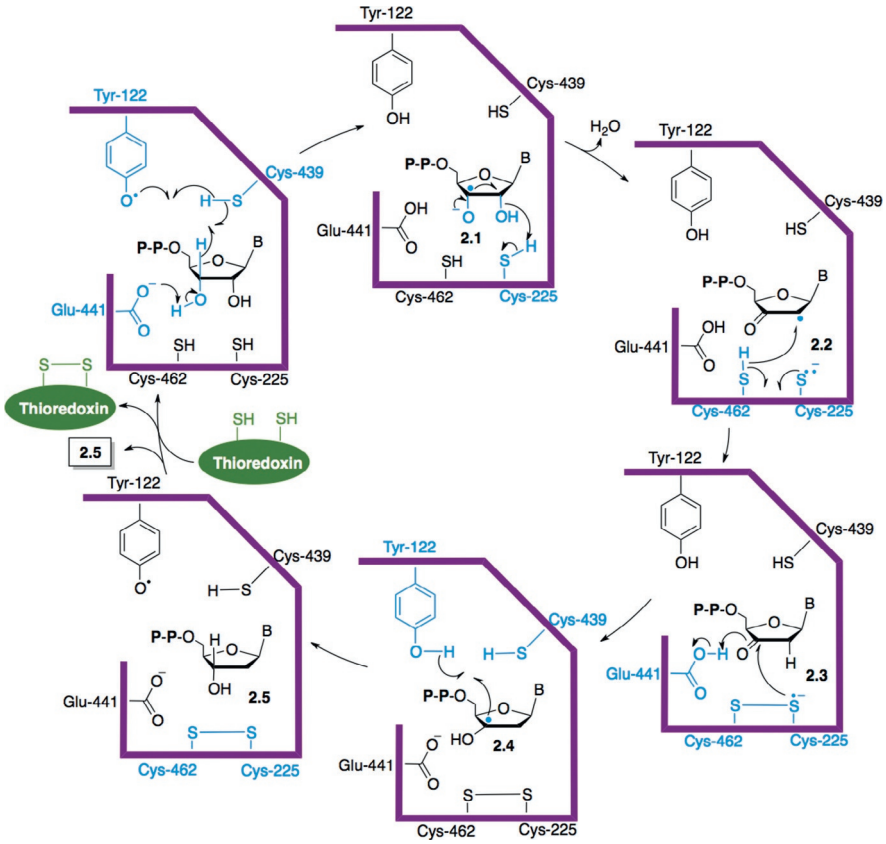
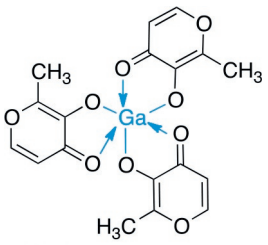



**FIGURE 2.7**

Catalytic cycle of ribonucleotide reductase.

form gallate anion ($Ga(OH)_4^-$) in blood, which is rapidly excreted in the urine. Gallium maltolate has the advantage of a lower renal toxicity, which is probably due to the fact that it becomes nearly entirely protein-bound in plasma. This drug has been tested in phase II clinical studies in patients with metastatic prostate cancer and refractory multiple myeloma. Interestingly, it has shown very good potential against metastasis, which has been attributed to its good transport into all kinds of cells by transferrin.[7]



Gallium maltolate

## 3.3 RADICAL SCAVENGERS

The best known inhibitor of ribonucleotide reductase is hydroxyurea (Hydrea®, Droxia®).[8] After oral administration, this compound is well absorbed and transported into cells, where it quenches the tyrosyl radical at the active site of ribonucleotide reductase, inactivating the enzyme (Figure 2.8).[9]

Nitric oxide, an important cell signaling molecule involved in many physiological processes, is one of the metabolic products of hydroxyurea, and its formation may contribute to the antitumor effect of the latter. In fact, nitric oxide is known to inhibit ribonucleotide reductase by itself, probably because it contains an unpaired electron and therefore it is able to quench the Tyr radical.[10] The mechanisms involved in the metabolic transformation of hydroxyurea into nitric oxide are multiple[11] and involve three-electron reduction processes. As an example, the mechanism of the peroxidase-mediated formation of nitric oxide from dismutation of the hydroxyurea radical to generate a nitroso derivative followed by hydrolysis of the latter is shown in Figure 2.9.

Hydroxyurea is primarily used in the management of myeloproliferative disorders, such as chronic granulocytic leukemia, polycythemia vera, and essential thrombocytosis, and is sometimes combined with other antitumor drugs such as the tyrosine kinase inhibitor imatinib.[12] Other applications of hydroxyurea include its use as a radiosensitizer and in AIDS therapy, in combination with didanosine. Hydroxyurea is also useful in the treatment of sickle cell anemia[13] because it eases the pain of the patients. This has been attributed to the previously mentioned generation of nitric oxide, a potent vasodilator.[14]

Thiosemicarbazones, represented by triapine, are another important class of inhibitors of ribonucleotide reductase. Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone, 3-AP) is a very strong iron chelator, and the iron chelate is probably the active species that quenches the active site

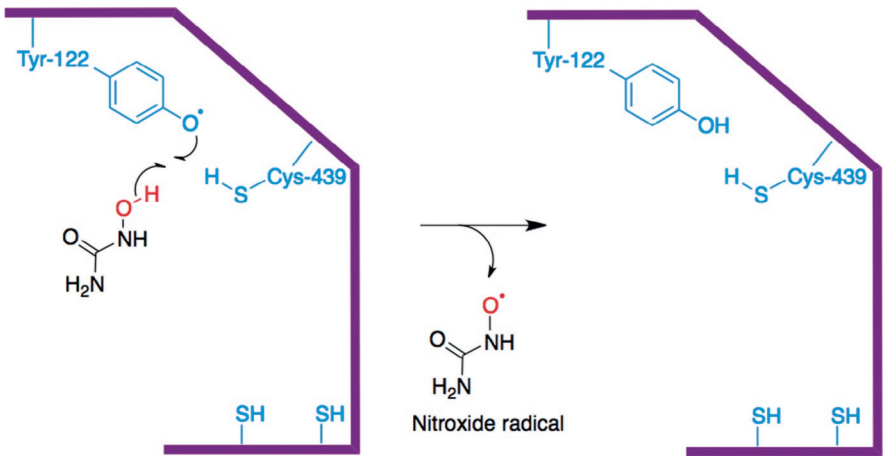



**FIGURE 2.8**

Mechanism of RNR inhibition by hydroxyurea.

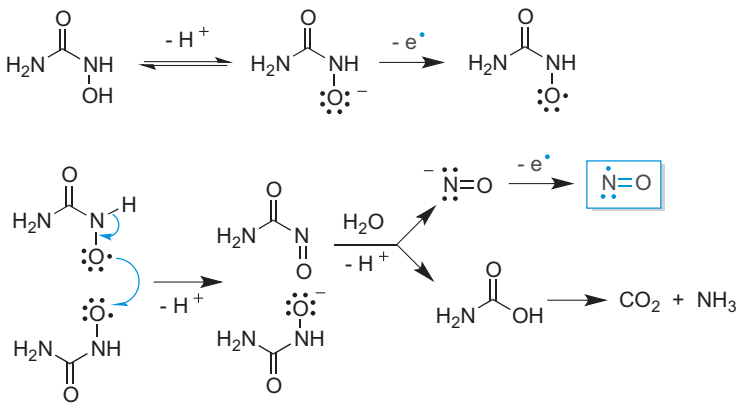
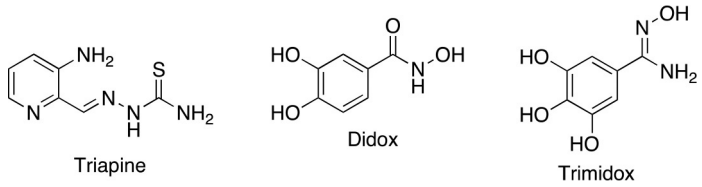


**FIGURE 2.9**

Mechanism of the peroxidase-mediated formation of nitric oxide from hydroxyurea.

tyrosyl radical of ribonucleotide reductase. 3-AP is a broad-spectrum anticancer agent[15] that has undergone phase I and II clinical studies for a variety of cancers, including solid tumors,[16] metastatic breast cancer,[17] and, in combination with cisplatin, locally advanced cervical cancer.[18]

Hydroxamic acid derivatives such as didox and trimidox are also RNR inhibitors. Didox, which is one of the most potent known inhibitors of the enzyme, has been recommended as a free radical scavenger to be used in combination with doxorubicin in order to lower its cardiotoxicity while enhancing its anticancer activity.[19] Trimidox was initially considered as an anticancer agent,[20] but it is employed mainly as an antibacterial agent for veterinary use.



Triapine Didox Trimidox

## 3.4 SUBSTRATE ANALOGS AS RIBONUCLEOTIDE REDUCTASE INHIBITORS

Ribonucleotide reductase substrate analogs are normally modified at C-2′, which is the position that undergoes reduction in the natural substrate. Many of these compounds bind covalently to the enzyme.

Tezacitabine (FmdC) is a nucleoside prodrug that shows a dual mechanism of action. Following intracellular phosphorylation, the tezacitabine diphosphate irreversibly inhibits ribonucleotide reductase, whereas the tezacitabine triphosphate can be incorporated into DNA during replication or repair, resulting in DNA chain termination.[21]