Cancer Chemotherapy and Biotherapy: Principles and Practice

SECOND EDITION

EDITED BY

Bruce A. Chabner, M.D.
Chief, Hematology/Oncology
Clinical Director
Massachusetts General Hospital Cancer Center
Boston, Massachusetts

Dan L. Longo, M.D.
Director, Biological Response Modifiers Program
Division of Cancer Treatment
National Cancer Institute
Frederick, Maryland

Lippincott - Raven
PUBLISHERS
Philadelphia • New York

Library of Congress Cataloging-in-Publications Data

Cancer chemotherapy and biotherapy : principles and practice / edited by Bruce A. Chabner, Dan L. Longo — 2nd ed.
p. cm.
Rev. ed. of: Cancer chemotherapy.
Includes bibliographical references and index.
ISBN 0-397-51418-2 (hard : alk. paper)
1. Cancer—Chemotherapy. 2. Cancer—Immunotherapy. I. Longo, Dan L (Dan Louis), 1949– . II. Cancer chemotherapy.
[DNLM: 1. Neoplasms—drug therapy. 2. Biological Products—therapeutic use. 3. Antineoplastic Agents—therapeutic use. 4. Chabner, Bruce. QZ 267 C21515 1996]
RC271.C5C32219 1996
616.99'4061—dc20
DNLM/DLC
for Library of Congress 95-38920
CIP

The material contained in this volume was submitted as previously unpublished material, except in the instances in which credit has been given to the source from which some of the illustrative material was derived.

Great care has been taken to maintain the accuracy of the information contained in the volume. However, neither Lippincott–Raven Publishers nor the editors can be held responsible for errors or for any consequences arising from the use of the information herein.

The authors and publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accord with current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new or infrequently employed drug.

Materials appearing in this book prepared by individuals as part of their official duties as U.S. Government employees are not covered by the above-mentioned copyright.

9 8 7 6 5 4 3 2 1

Cancer Chemotherapy and Biotherapy, second edition,
edited by Bruce A. Chabner and Dan L. Longo.
Lippincott–Raven Publishers, Philadelphia ©1996

CHAPTER 6

Antifolates

Edward Chu and Carmen J. Allegra

The folate-dependent enzymes represent attractive targets for antitumor chemotherapy because of their critical role in the synthesis of the nucleotide precursors of DNA (Fig. 6-1). In 1948, Farber et al.[1] were the first to show that aminopterin, a 4-amino acid analogue of folic acid, could inhibit the proliferation of leukemic cells and produce remissions in acute leukemia. Their findings ushered in the era of antimetabolite chemotherapy and generated great interest in the antifolate class of agents. Since then, the clinical value of antifolate compounds has been proven in the treatment of the leukemias, breast cancer, head and neck cancer, lymphomas, and choriocarcinoma.[2] Their clinical application also has been extended to the treatment of nonneoplastic disorders, including rheumatoid arthritis,[3] graft-versus-host disease following bone marrow transplantation,[4] psoriasis,[5] bacterial and plasmodial infections,[6] and opportunistic infections associated with the acquired immunodeficiency syndrome (AIDS).[7] It is fair to state that this class of agents is the best understood and most versatile of all the cancer chemotherapeutic drugs (Table 6-1).

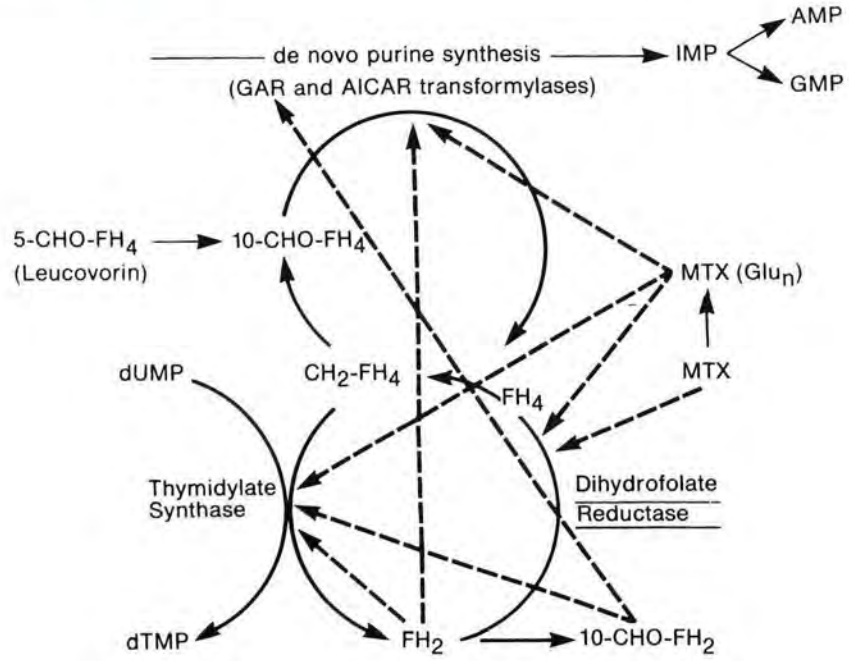
MECHANISM OF ACTION

Substitution of an amino group for the hydroxyl at the 4-position of the pteridine ring is the critical change in the structure of antifolate compounds that leads to their antitumor activity (Fig. 6-2). This change transforms the molecule from a substrate to a tight-binding inhibitor of dihydrofolate reductase (DHFR), a key enzyme in intracellular folate homeostasis. The critical importance of DHFR stems from the fact that folic acid compounds are active as coenzymes only in their fully reduced tetrahydrofolate form. There are two specific tetrahydrofolates that play essential roles as one-carbon carriers involved in the synthesis of DNA precursors. 10-Formyltetrahydrofolate provides its one-carbon group for the de novo synthesis of purines in reactions mediated by glycineamide ribonucleotide (GAR) transformylase and aminoimidazole carboxamide ribonucleotide (AICAR) transformylase. A second cofactor, 5, 10-methylenetetrahydrofolate (CH_2FH_4), donates its one-carbon group to the reductive methylation reaction converting deoxyuridylate (dUMP) to thymidylate (dTMP) (see Fig. 6-1). In addition to yielding a one-carbon group, 5, 10-methylenetetrahydrofolate is oxidized to dihydrofolate, which must then be reduced to tetrahydrofolate by the enzyme DHFR in order for it to rejoin the pool of active reduced folate cofactors. In actively proliferating tumor cells, inhibition of DHFR by methotrexate (MTX) (see Fig. 6-2) or other 2,4-diamino folates may lead to an accumulation of folates in the inactive dihydrofolate form, with variable depletion of reduced folates.[8–14] Folate depletion, however, does not fully account for the metabolic inhibition associated with antifolate treatment, since the critical reduced folate pools are relatively preserved even in the presence of cytotoxic concentrations of MTX. Additional factors may contribute to MTX-associated cytotoxicity, including metabolism of the parent compound to polyglutamated derivatives and the accumulation of dihydrofolate and 10-formyldihydrofolate polyglutamates as a consequence of DHFR inhibition.[8,9,15–17] MTX polyglutamates, dihydrofolate polyglutamates, and 10-formyldihydrofolate metabolites represent potent direct inhibitors of the folate-dependent enzymes of thymidylate and purine biosynthesis.[18–23] Thus inhibition of DNA biosynthesis by 2,4-diamino folates is a multifactorial process consisting of both partial depletion of reduced folate substrates and direct inhibition of folate-dependent enzymes. The relative role of each of these mechanisms in determining antifolate-associated metabolic inhibition may depend on specific cellular factors that vary among different cancer cell lines and tumors.

CHEMICAL STRUCTURE

Various heterocyclic compounds with the 2,4-diamino structural configuration have antifolate activity and include pyrimidine analogues such as pyrimethamine and trimethoprim[20–29] (see Fig. 6–2), classic pteridines such as aminopterin and methotrexate,[2] and compounds with replacement of the nitrogen at either the 5, 8, or 10 position with a carbon atom, such as the quina-

Figure 6-1. Sites of action of MTX, its polyglutamated metabolites [MTX(Glu_n)], and folate by-products of the inhibition of DHFR, including dihydrofolate (FH_2) and 10-formyldihydrofolate (10-CHO-FH_2). Also shown are 5,10-methylenetetrahydrofolate (CH_2-FH_4), the folate cofactor required for thymidylate synthesis, and 10-formyltetrahydrofolate (10-CHO-FH_4), the required intermediate in the synthesis of purine precursors. (From Chabner et al. In: DeVita VT, Hellman S, Rosenberg SA, eds. Cancer principles and practice of oncology. Philadelphia: JB Lippincott, 1989:349.)



Compound	Inhibits
MTX	Dihydrofolate reductase
MTX (Glu_n)	Dihydrofolate reductase Thymidylate synthase AICAR transformylase GAR transformylase
FH_2 (Glu_n)	Thymidylate synthase AICAR transformylase GAR transformylase
10-CHO-FH_2 (Glu_n)	Thymidylate synthase GAR transformylase

zolines (trimetrexate, piritrexim)[24–26] and 10-ethyl-10-deazaaminopterin (10-EDAM, Edatrexate).[27] Compounds with preservation of the benzoylglutamate terminal group are transported by a folate-specific system in the cell membrane, while those lacking a terminal glutamate do not require active transmembrane transport and have activity against MTX-resistant cells that lack the folate transporter.[28] Recently, investigators have designed antifolate analogues directed at targets other than DHFR, including those folate-dependent enzymes required for the de novo synthesis of purines and thymidylate. A host of potent thymidylate synthase inhibitors such as 10-propargyl-5,8-dideazafolate (PDDF, CB3717)[29] and closely related compounds ZD1694,[30] LY231514,[31] 1843U89,[32] and 5,8-dideazatetrahydrofolic acid (DDATHF),[33] an inhibitor of GAR transformylase, have been developed recently. Although each of these analogues has unique structural features distinct from MTX with equal or greater potency for inhibiting DHFR or other folate-dependent enzymes, none has yet replaced MTX in the clinic. This is

Table 6-1. Key Features of Methotrexate

Mechanism of action:	Inhibition of dihydrofolate reductase leads to partial depletion of reduced folates. Polyglutamates of methotrexate and dihydrofolate inhibit purine and thymidylate biosynthesis.
Metabolism:	Converted to polyglutamates in normal and malignant tissues. 7-Hydroxylation in liver.
Pharmacokinetics:	$t_{1/2}\alpha$ = 2–3 h; $t_{1/2}\beta$ = 8–10 h
Elimination:	Primarily as intact drug in urine.
Drug interactions:	1. High dose toxicity to normal tissues rescue by leucovorin. 2. L-Asparaginase blocks toxicity and antitumor action. 3. Pretreatment with methotrexate increases 5-fluorouracil and araC nucleotide formation. 4. Nonsteroidal anti-inflammatory agents decrease renal clearance and increase toxicity.
Toxicity:	1. Myelosuppression 2. Mucositis, gastrointestinal epithelial denudation 3. Renal tubular obstruction and injury 4. Hepatotoxicity 5. Pneumonitis 6. Hypersensitivity 7. Neurotoxicity
Precaution:	1. Reduce dose in proportion to decrease in creatinine clearance. 2. Do not administer high-dose methotrexate to patients with abnormal renal function. 3. Monitor plasma concentrations of drug and hydrate patients during high-dose therapy (see Tables 6-2 and 6-4).

primarily because of greater familiarity with both the cytotoxic activity and host toxicity patterns of MTX and the current lack of evidence that these analogue compounds with the possible exception of the thymidylate synthase inhibitors, have either improved therapeutic efficacy or a different spectrum of clinical activity.

CELLULAR PHARMACOLOGY AND MECHANISMS OF RESISTANCE

In this section the sequence of events that lead to the cytotoxic action of MTX will be considered, beginning with drug movement across the cell membrane, followed by its intracellular metabolism to the polyglutamate derivatives, binding to dihydrofolate reductase and other folate-dependent enzymes, effects on intracellular folates, and finally, inhibition of DNA synthesis. Each of these steps plays an essential role in determining the ultimate clinical efficacy and toxicity of MTX and other antifolate compounds.

Transmembrane Transport

The movement of MTX and other antifolates across cell membranes has received much attention because of the potential role of transport abnormalities in the development of clinical drug resistance (Fig. 6-3). MTX enters cells by an energy-dependent, temperature-sensitive, and concentrative process that likely depends on the function of specific intramembrane protein(s).[34–39] This mechanism is anion-dependent and glucose-insensitive.[40–43] For a comprehensive review of the cellular transport of folates, the reader is referred to the recent article by Antony.[44]

The membrane carrier responsible for MTX transport in mammalian cells also transports the naturally occurring reduced folates, including the rescue agent 5-formyltetrahydrofolate (leucovorin).[38,43–47] Thus MTX and physiologic folates compete for cellular entry. In addition, through a process known as *heteroexchange*, free intracellular MTX is forced to efflux from cells when high concentrations of extracellular reduced folate cross the cell membrane.[48]

The influx carrier for MTX is comprised of at least two different systems which include (1) a carrier often referred to as the reduced-folate carrier with a higher affinity for reduced folates and MTX (affinity constant = 0.7 to 6 μ*M*) when compared with folic acid (affinity constant = 200 μ*M*) and (2) a hydrophobic membrane-associated folate-binding protein(s) (human folate receptor, hFR) that has a 10- to 30-fold higher affinity for folic acid and the reduced folates (nanomolar range) than for MTX.[34–39,47,49–52] MTX polyglutamates demonstrate a 100-fold increased affinity for the folate-binding protein when compared with the monoglutamate form of MTX.[53] Whether these two transport systems are directly interrelated or function separately awaits the complete molecular characterization of each of these transport systems. Recently, a process referred to as *potocytosis*, a mechanism distinct from receptor-mediated endocytosis, was found to be associated with folate-binding proteins.[54–56] This mechanism has been used to describe the accumulation of binding proteins in distinct regions of the cell membrane known as *caveola* that form intracellular vesicles containing protein-bound folates. Presumably, folates are released from their hFRs through changes in pH that occur within the caveola. Once released from the hFRs in the caveola, the folates may then enter the intracellular space via a specific membrane carrier, perhaps representing the reduced-folate car-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.