HEMATOLOGY

Basic Principles and Practice

Edited by

Ronald Hoffman, M.D.
Bruce Kenneth Wiseman Professor of Medicine, Professor of Pathology, and
Chief, Hematology/Oncology Section, Department of Medicine,
Indiana University School of Medicine,
Indianapolis, Indiana

Edward J. Benz, Jr., M.D.
Chief, Section of Hematology, Associate Chairman for Academic Affairs,
Department of Internal Medicine, Professor, Departments of Internal Medicine
and Human Genetics, Yale University School of Medicine;
Attending Physician, Yale-New Haven Hospital,
New Haven, Connecticut

Sanford J. Shattil, M.D.
Professor, Departments of Medicine and Pathology and Laboratory Medicine,
University of Pennsylvania School of Medicine;
Chief, Hematology-Oncology Section, Hospital of the University of Pennsylvania,
Philadelphia, Pennsylvania

Bruce Furie, M.D.
Professor, Departments of Medicine and Biochemistry,
Tufts University School of Medicine;
Chief, Division of Hematology-Oncology, Department of Medicine,
New England Medical Center Hospital,
Boston, Massachusetts

Harvey J. Cohen, M.D., Ph.D.
Professor of Pediatrics, and Chief, Division of Pediatric Hematology/Oncology,
Department of Pediatrics and the Cancer Center,
University of Rochester School of Medicine and Dentistry,
Rochester, New York

Churchill Livingstone
New York, Edinburgh, London, Melbourne, Tokyo

Library of Congress Cataloging-in-Publication Data

Hematology : basic principles and practice / edited by Ronald Hoffman
. . . [et al.].
p. cm.
Includes bibliographical references and index.
ISBN 0-443-08643-5
1. Hematology. I. Hoffman, Ronald, date.
[DNLM: 1. Hematologic Diseases——diagnosis. 2. Hematologic Diseases—therapy. WH 100 H48745]
RC633.H434 1991
616.1′5—dc20
DNLM/DLC
for Library of Congress 90-15112
CIP

Distributed in the United Kingdom by Churchill Livingstone, Robert Stevenson House, 1–3 Baxter's Place, Leith Walk, Edinburgh EH1 3AF, and by associated companies, branches, and representatives throughout the world.

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Printed in the United States of America

First published in 1991 7 6 5 4 3

Pharmacology of Antineoplastic Agents and Mechanisms of Multidrug Resistance

52

Antonio C. Buzaid and Ed Cadman

HISTORICAL ASPECTS

The vesicant properties of sulfur mustard have been known for more than 100 years. It was only in 1919, however, that Krumbhaar and Krumbhaar observed that poisoning by sulfur mustard produced leukopenia, aplasia of the bone marrow, marked decrease in lymphoid tissue, and ulceration of the gastrointestinal tract. These findings prompted Goodman and associates to test whether nitrogen mustard could be used therapeutically.[1] Following successful animal studies, clinical trials were launched in 1942, which ushered in the modern era of chemotherapy.

In 1948 methotrexate became available, and shortly thereafter 5-fluorouracil was synthesized. In 1955 the first chemotherapeutic cure using methotrexate as a treatment for metastatic choriocarcinoma was recorded. During the 1960s investigation of certain medicinal plants resulted in the development of the Vinca alkaloids and podophyllotoxin derivatives. Late in that decade DeVita and colleagues showed that combinations of chemotherapeutic drugs produced better results than single-agent therapy.[2] Since then the incorporation of daunorubicin, doxorubicin, and cytosine arabinoside in the oncologic armamentarium has resulted in a significant impact in the curability of many non-Hodgkin's lymphomas and acute leukemias. Clinical trials since about 1970 have produced a number of useful chemotherapeutic regimens.

In this chapter we review the pharmacology of antineoplastic drugs, with special focus on the cytotoxic agents employed in the treatment of hematologic malignancies. In addition, we will provide an overview of their mechanisms of action as well as potential strategies designed to overcome drug resistance.

CELLULAR KINETICS AND TUMOR GROWTH

At any given time only a portion of the cells in a tumor are actively dividing; this subset of cells is called the growth fraction. When a malignancy first arises, most of the tumor cells are dividing, and the growth fraction is high. As the tumor grows, a larger proportion of the cells become inactive and assume a resting state. The decline in growth fraction may be due to restrictions of space, nutrient availability, and blood supply. This pattern of growth does not follow a classical exponential growth curve and is best described by the Gompertz equation. The growth fraction depends also on the type of tumor with values ranging from less than 10 percent for some adenocarcinomas to greater than 90 percent for some lymphomas.[3] This concept is of great importance since most chemotherapeutic agents are more effective against dividing cells than against resting cells.

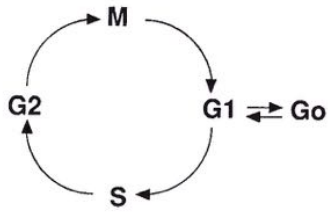

Fig. 52-1. Phases of the cell cycle. Go, resting phase (nonproliferation of cells); G1, pre-DNA synthetic phase (12 hours to a few days); S, DNA synthesis (usually 2 to 4 hours); G2, post-DNA synthesis (2 to 4 hours; cells are tetraploid in this stage); M, mitosis (1 to 2 hours).

A schematic presentation of the events occurring during the cell cycle is shown in Figure 52-1. Cytotoxic agents can be divided into phase-specific and phase-nonspecific according to their predominant effect on the cell cycle.

1. *Phase-nonspecific agents* are effective in any phase of the cell cycle. Agents that fall into this category usually have a linear dose-response curve (i.e., the greater the dose administered, the greater the fraction of cell kill). They are divided into two subgroups:
   a. *Cycle-specific agents* kill cells that are proceeding through the cell cycle independently of whether the cell is in G1, G2, S, or M phase (e.g., alkylating agents, cisplatin).
   b. *Cycle nonspecific agents* kill nondividing cells (e.g., steroids and antitumor antibiotics except bleomycin).
2. *Phase-specific agents* are effective only if present during a certain phase of the cell cycle. Within a certain dose range agents of this category show no increase in cell kill with further increase in dose. If the drug is maintained over a period of time, however, more cells will enter the specific lethal phase of the cycle and be killed. Examples include L-asparaginase (G1 phase), antimetabolites (S phase), and Vinca alkaloids (M phase).

Chemotherapeutic agents are not completely specific and affect normal as well as neoplastic cells. This effect is most pronounced with rapidly proliferating cells, such as the mucosa of the gastrointestinal tract and the bone marrow. This limits dose escalation and usually determines the maximum tolerated dose.

TUMOR HETEROGENEITY

Cancer has been shown to be a clonal disease, (i.e., the cancer cells descend from a single progenitor cell). However, as cancers progress they become markedly heterogeneous. Within a single

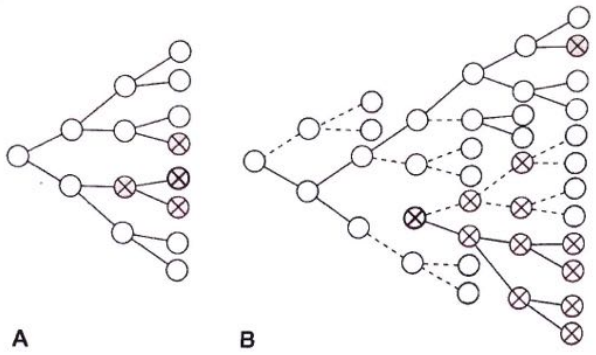

Fig. 52-2. "Growth trees" of stem cell compartments up to eight cells for (**A**) a death rate of 0 and (**B**) a death rate of 0.6. A circle that branches into two circles indicates the division of a stem cell to form two new stem cells; the gray circles indicate that the division produced two differentiated cells. Dotted lines indicate ancestries in which all the cells have subsequently become differentiated (or died), and solid lines indicate continued stem cell proliferation. Resistance (pink circles) occurs at the fifth branch (line connecting two viable stem cells) in cells that are sensitive (not resistant). All cells resulting from the division of a resistant cell are themselves resistant. In **A** it can be seen that this leads to three cells being resistant; in **B**, the comparatively greater number of branches leads to five of the eight cells being resistant. This illustrates the enhancement of resistance caused by cellular differentiation (or death). *Note:* resistance is assumed to occur deterministically rather than randomly in order to simplify illustration, but it would not be expected to do so in reality. (Modified from Goldie and Coldman,[6] with permission.)

tumor this heterogeneity is expressed as variations in histopathology, cytogenetics, expression of surface antigens, growth rate, metastatic potential, and more importantly, sensitivity to cytotoxic agents. The major factor responsible for this heterogeneity is spontaneous mutation. Tumors are also heterogeneous in their supply of nutrients and oxygen, factors that may further increase their genetic instability.[4,5] The overall growth of a tumor is dictated by the number of cell doublings, its growth fraction, and the death rate of the cancer cells. For example, in a given tumor (Fig. 52-2A) for which a constant mutation rate and a death rate of zero are assumed, the higher the number of cell doublings, the larger the tumor, the larger the number of mutations, and thus the higher the chance of having chemotherapy-resistant clones. If a constant mutation rate is assumed in a tumor with a high death rate (Fig. 52-2B), many more cell doublings and therefore many more mutations must occur for the tumor to reach the same size.[6] The latter situation applies to tumors that are slow-growing, apparently because the rate of cell loss is high. Thus, at the time that these slow-growing tumors are clinically detectable, they have already undergone multiple mutations and have a large number of cells that are resistant to virtually all available anticancer agents. In simple terms, one can envision a patient with a bulky and slow-growing tumor as actually having multiple different cancers. Consequently, current chemotherapy is seldom successful in patients with this type of condition.

The mathematical model of Goldie and Coldman[6] allows one to develop a better intuitive understanding of the events that occur during the treatment of cancer. This model substantiates the concept of dose intensity developed by Hryniuk and Bush[7] and also validates the importance of employing multiple cytotoxic drugs instead of single drugs to decrease the development of resistance. In addition, this hypothesis has resulted in the use of alternating non-cross-resistant regimens. This approach has recently gained wide popularity. However, since most chemotherapy regimens presently available are at best only partially non-cross-resistant—as, for example MOPP (mechlorethamine, oncovin, procarbazine, and prednisone) alternating with ABVD (Adriamycin, bleomycin, vinblastine, and dacarbazine) the therapeutic value of this strategy has not been properly tested

DEVELOPMENT OF CHEMOTHERAPEUTIC AGENTS

Chemotherapeutic agents may be developed (1) by synthetic procedures using new biochemical and pharmacologic concepts and structure-activity relationships; (2) from natural sources (e.g., plant extracts, microbial fermentation, and marine organisms); and (3) by examining new synthetic compounds made for other purposes.

Screening for Antitumor Activity

The concept of screening agents for antitumor activity is based on the rationale that an appropriate bioassay may reliably indicate activity against human cancers. At the National Cancer Institute the algorithm shown in Figure 52-3 has been used since 1972. Modifications are frequently made in an attempt to increase the predictive value of the screening panel. After drug acquisition and formulation, the first step in the screening process is evaluation of anticancer activity against the mouse P388 leukemia model. Active drugs are subjected to a broader testing panel, which includes many mouse tumors as well as human tumors xenografted in nude mice. If no activity is seen in the P388 leukemia model, the drug is cross-checked against human tumors grown in soft agar and by the subrenal capsule assay. Drugs that are not active in the P388 leukemia model but show activity in other biologic or biochemical systems will also undergo evaluation in the broader testing panel. If anticancer activity is observed either in the broader panel or in the soft agar or subrenal capsule assay, the drug enters preclinical toxicologic testing. Despite many modifications, it is not known how well this current system identifies potentially active antineoplastic agents.

Clinical Trials

Following extensive toxicologic studies, the new drug usually enters clinical trials in humans, which are divided into four phases. Phase I trials are designed to determine the toxicity profile, the maximum tolerated dose (MTD), and pharmacologic data; the determination of anticancer activity is a secondary goal. Patients with refractory cancers are candidates for phase I trials. The initial dose employed is usually 10 percent of the lethal dose (LD_{10}) found in rodents during the preclinical studies. This dose is progressively escalated, generally according to a modified Fibonacci scale (Table 52-1). At least three patients are usually enrolled and evaluated at each dose level. Once the MTD and toxicity profile are determined, the drug enters phase II studies, which are designed primarily to determine the efficacy of the new compound in different types of cancer, generally the 7 to 10 most common ones. Phase III trials generally compare the efficacy of the new drug in a randomized fashion with the existing "standard therapy." In phase IV trials, usually conducted after the drug has

Table 52-1 Modified Fibonacci Dose Escalation Scheme Used in Phase I Trials

Drug Dose (mg/m^2)	Percent Increment above Prior Dose Level
n	(Initial dose level)
2n	100
3.3n	65
5n	51
7n	40
9n	28
12n	33

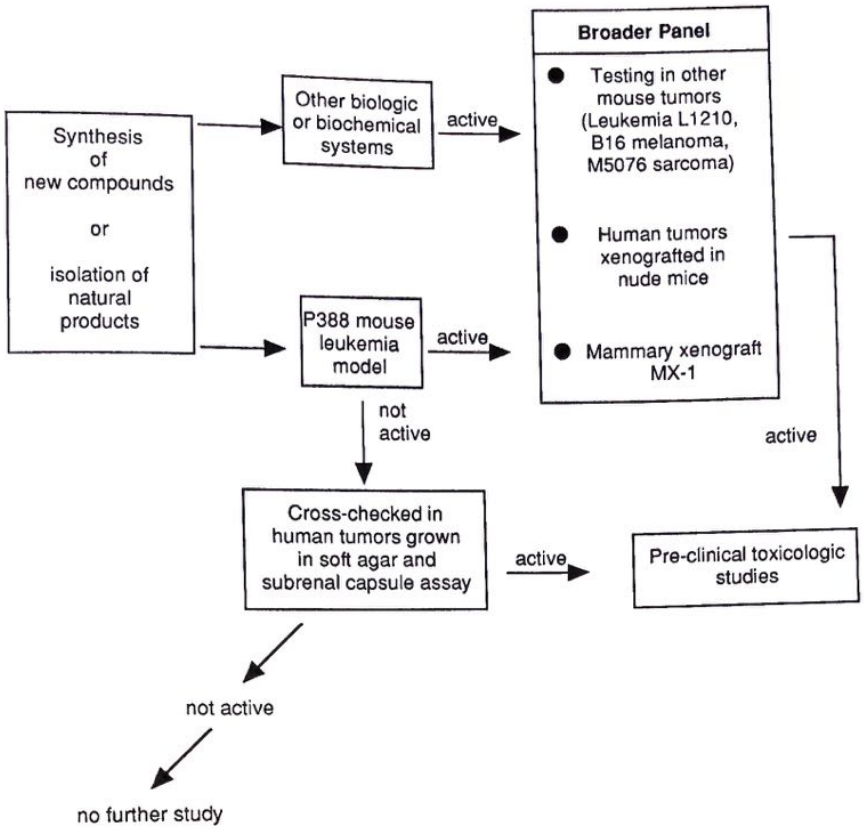

Fig. 52-3. Algorithm used for drug screening at the National Cancer Institute.

been marketed, the drug is combined with other treatment modalities (e.g., radiation and/or surgery) and compared in a randomized study with the standard therapy.

PHARMACOLOGY OF CHEMOTHERAPEUTIC AGENTS

A number of fundamental molecular processes must take place for cells to proliferate. DNA must be replicated without error, a process that requires an appropriate supply of purine and pyrimidine nucleotides and multiple enzymes such as DNA polymerases. These enzymes are produced from complementary RNA synthesized from an intact DNA template. The RNA is translated into proteins through a complex polymerization reaction, which takes place on the ribosomes in the cell cytoplasm. After the cells have replicated their DNA, they undergo mitosis. Cytotoxic agents interfere with one or more of these essential cellular processes (Fig. 52-4).

Cytotoxic agents have been classically divided into alkylating agents, plant alkaloids, antitumor antibiotics, antimetabolites, and a miscellaneous group. There are presently more than 40 standard (i.e., commercially available) chemotherapeutic agents. The pharmacology of those agents used in the treatment of hematologic malignancies is presented below.

Alkylating Agents

Alkylating agents contain alkyl groups that bond covalently with nucleophilic substances of the DNA and/or proteins associated with the DNA. On the basis of their chemical structure the alkylating agents are divided into the five groups shown in Table 52-2.

General Mechanism of Action

The cytotoxic as well as the mutagenic effects of the alkylating agents are directly related to the alkylation and disruption of DNA. Figure 52-5 shows the various mechanisms by which mechlorethamine (nitrogen mustard) may alkylate the DNA. While mechlorethamine is used here to illustrate the effects of the alkylating agents on the DNA, the same basic mechanisms apply

Table 52-2. Alkylating Agents

Nitrogen mustards
Mechlorethamine
Cyclophosphamide
Ifosfamide
Chlorambucil
Melphalan
Ethylenimines
Thiotepa
Hexamethylmelamine[a]
Alkylsulfonates
Busulfan
Nitrosoureas
Carmustine
Lomustine
Streptozocin
Triazines
Dacarbazine (DTIC)

[a] Investigational drug.

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