

AMERICAN CANCER SOCIETY TEXTBOOK OF

C L I N I C A L
ONCOLOGY

ARTHUR I. HOLLEB, MD

DIANE J. FINK, MD

GERALD P. MURPHY, MD



The American Cancer Society, Inc., Atlanta, GA 30329

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Published 1991. First edition 1991

Printed in the United States of America

94 93 92 5 4 3 2

Library of Congress Cataloging in Publication Data

American Cancer Society textbook of clinical oncology / Arthur I.
Holleb, Diane J. Fink, Gerald P. Murphy.

p. cm.

Includes bibliographical references.

Includes index.

ISBN 0-944235-07-7

1. Cancer. 2. Oncology. I. Holleb, Arthur I., 1921-
II. Fink, Diane J. III. Murphy, Gerald P. IV. American Cancer
Society. V. Title: Textbook of clinical oncology.

(DNLM: 1. Neoplasms. QZ 200 A5127)

RC261.A677 1991

616.99'4—dc20

DNLM/DLC

91-4642

for Library of Congress

CIP

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PREFACE

The *American Cancer Society Textbook of Clinical Oncology* is the product of the combined efforts of 50 of the leading cancer experts in the United States. The Society assembled this distinguished group of authors to create a new, more comprehensive text for medical students and for physicians working in, or interested in, oncology; a text that addresses not only the treatment of the disease, but also the related issues that are keys to a multidisciplinary approach to cancer care.

In the time that has elapsed since the publication of the last edition of this text's predecessor, *Clinical Oncology: A Multidisciplinary Approach*, the body of knowledge relative to cancer has grown dramatically. The range of issues affecting the cancer patient has expanded, and there has been an explosion in biological knowledge as it relates to cancer treatment. A medical text was needed that would take into account this rapid growth in the science of treatment as well as how clinical issues relate to research. Thus, the American Cancer Society felt that a more comprehensive treatment of the various forms of the disease was required rather than a revision of our former oncology text. For this reason, the American Cancer Society undertook publication for the first time of its own new textbook, one that would embrace the entire body of knowledge relative to oncology and the issues critical to cancer control.

In addition to timely, in-depth discussions of the pathology, etiology, and nature of cancer, the scope of this new text has been broadened to reflect the increased specialization within medicine and the team approach to patient care. And, in keeping with the emerging understanding that health care must be a partnership between physicians and patients, theories of lifestyle factors and preventive measures have been addressed, as have the areas of social work, sexuality, and rehabilitation.

Because of the skill and knowledge of our authors and their broad base of experience in both research and clinical practice, it is our anticipation that the *American Cancer Society Textbook of Clinical Oncology* will be recognized as a definitive work in oncology, one that will be of great educational value to medical students, physicians, and nurses in the United States and elsewhere.

Arthur I. Holleb, M.D.
Diane J. Fink, M.D.
Gerald P. Murphy, M.D.

INTRODUCTION

Arthur I. Holleb, M.D.

*Arthur I. Holleb, M.D.
Consultant, Medical Affairs Department
American Cancer Society
Larchmont, New York*

As a professional education document, the purpose of this American Cancer Society book is to accomplish certain objectives by providing the reader with not only the basics about cancer, but also with up-to-date information about the disease from recognized experts in their respective fields.

The American Cancer Society traces its origins to 1913, when a group of 10 physicians and five laymen met in New York City and founded the American Society for the Control of Cancer. Its stated purpose at the time was to "disseminate knowledge concerning the symptoms, treatment, and prevention of cancer; to investigate conditions under which cancer is found; and to compile statistics in regard thereto." Later renamed the American Cancer Society, it is today one of the oldest and largest voluntary health agencies in the United States, comprising 2.2 million American volunteers united to conquer cancer through balanced programs of research, education, patient service, and rehabilitation.

The American Cancer Society, Inc. is organized as a National Society, with 57 chartered Divisions and 3,170 Units. A 206-member House of Delegates provides representation from the 57 geographic Divisions and additional representation on the basis of population. It elects, and is governed by, a Board of Directors of 124 voting members, half of whom are members of the medical or scientific professions.

The National Society is responsible for overall planning and coordination. It also provides technical help and materials to Divisions and Units, administers programs of research, medical grants, and clinical and nursing fellowships, and carries out public and professional education on the national level.

The 57 Divisions are governed by members, both medical and lay people, of Divisional Boards of Directors in all the states plus five metropolitan areas, the District of Columbia, and Puerto Rico. The Units are organized to cover the counties in the United States. There are thousands of community leaders who direct the Society's programs at this level.

The Society maintains its priorities and goals through activities developed by the departments of Research, Professional Education, Public Education, Public Information, Epidemiology and Statistics, Service and Rehabilitation, Public Affairs, and Crusade.

Details of every American Cancer Society program can be obtained by writing to the appropriate Department at the National Headquarters, located at 1599 Clifton Road, N.E., Atlanta, GA 30329.

Knowledge and understanding about cancer, its early detection, its prompt diagnosis, and its adequate treatment will assuredly lead to further increases in survival rates. Rehabilitation of the person treated for cancer will improve the quality of his or her life. Of course, the use of measures that can prevent cancer, whenever possible, are ideal goals. These are the objectives this book strives to accomplish.

Cancer is a group of diseases characterized by the uncontrolled growth of abnormal cells that spread from the anatomic site of origin. This spread, if uncontrolled, invades vital organs and results in death. However, many cancers can be cured if they are detected early and treated promptly; others can be controlled for many years with a variety of treatment approaches.

Cancer is most often treated by surgery, radiation therapy, chemotherapy, and hormones. More recently, immunotherapy was added to the therapeutic armamentarium. Cancer occurs at any age. It results in the death of more children ages 3 to 14 than any other disease. Also, it strikes more frequently in older people. According to the American Cancer Society, in the 1980s there were more than 4.5 million deaths due to cancer, almost 9 million new cancer cases, and about 12 million people under medical care for cancer in the United States.

More than 6 million Americans alive today have had a history of cancer; most, but not all, of the 3 million of them who were diagnosed five or more years ago can be considered cured. "Cure" is defined as having no evidence of recurrent cancer while simultaneously having the same life expectancy as a person who never had cancer. For most forms of cancer, five years after treatment with no signs of recurrence is a good statistical mark for continued survival, although there still can be an attrition rate beyond that time. In fact, for some of the more aggressive forms of cancer which frequently are fatal in a short period of time, a 3-year survival may be considered approaching a cure. Others cancers may require much longer periods of time after treatment to be considered cured.

During the year of this text's publication, more than 1 million people will be diagnosed as having cancer. This estimate of incidence is based upon data from the National Cancer Institute's Surveillance, Epidemiology and End Result (SEER) Program (1984-1986). Nonmelanoma skin cancer and carcinoma *in situ* are not included in the statistics. For example, more than 600,000 cases of nonmelanoma skin cancer occur annually.

At the turn of this century very few people with major cancers had much hope of long-term survival. More than 50 years ago, in the 1930s, less than 1 in 5 persons with cancer was alive five years after treatment. In the 1940s it was 1 in 4, and in the 1960s it was 1 in 3.

Today, 40%, or 4 out of 10 persons who develop cancer, will be alive five years after treatment. The improvement from 1 in 3, to 4 in 10 represents 69,000 people in 1990 alone. This 40% figure is the "observed" survival rate. Taking into consideration normal life expectancy (e.g., dying of heart disease, accidents, and so forth) 50% will be alive five years after treatment. This is the "relative" survival rate, a more accurate measure of success in the treatment of cancer.

In 1989 about 42,000 people with cancer died who might have been saved by earlier detection and prompt treatment. This figure emphasizes the need for more programs of public and professional education. In 1989, an estimated 510,000 people in the United States died of cancer—1,400 people every day, or about one death from cancer every minute.

Except for lung cancer, the age-adjusted cancer death rates for major anatomic sites are leveling off, and in some cases declining. "Age-adjusted" is a method used to make valid statistical comparisons by assuming the same age distribution among different groups being compared.

Not all cancers can be prevented using our present state of knowledge; however, some can. Most cancers of the lung are caused by cigarette smoking, and most skin cancers are caused by excessive exposure to direct sunlight. Those cancers caused by occupational and/or environmental factors can be prevented by eliminating or reducing contact with carcinogenic agents.

In the chapters that follow, the reader will be presented with information about how cancer develops; the principles of cancer biology; general trends in screening asymptomatic people; diagnosis and treatment; approaches to modern medical technology; the principles of oncologic specialties; prevention and causation of cancer; the special roles of smoking, nutrition, and viruses; specific types and anatomic sites of cancer; medical emergency situations that occur in cancer patients; AIDS-related cancer; rehabilitation, supportive care, pain control, psychosocial management, and sexuality issues; the remarkable recent advances in diagnostic imaging; and other topics of vital interest to the student of oncology.

No textbook can be complete unto itself; therefore the reader is encouraged to make use of the extensive lists of references following each chapter to expand on the information presented.

Cancer control is a rational pursuit. Considerable progress has been made over the years for many types of cancer and even more progress can be anticipated in the future. Basic scientists are describing the present as the "golden years of cancer research"—an era filled with more promise than at any other time in the past.

The more we educate people about cancer, the more it will come out of the closet. Less and less will there be a cry of anger and resentment against the heavens or fate; the "Why me?" or the "Why has God done this to me?" syndrome will fade. That sense of shame and self-blame when cancer strikes will lessen, and cancer will be looked upon as an illness like any other, not as a social disgrace or stigma.

Oncologists are designing treatment so that patients can live with dignity and respect, not merely exist. More often, the patient is being given what I like to call an "ego prosthesis." In the future, there will be greater and more open communication about cancer between patients, families, and physicians; more truth telling, fewer charades and conspiracies of silence.

It is our hope that this textbook will assist physicians in their communications with other physicians and with their cancer patients, and result in a higher level of mutual understanding.

PRINCIPLES OF MEDICAL ONCOLOGY

Michael R. Cooper, M.D.

M. Robert Cooper, M.D.

*Michael R. Cooper, M.D.
Senior Clinical Investigator
Clinical Pharmacology Branch
National Cancer Institute
Bethesda, Maryland*

*M. Robert Cooper, M.D.
Professor of Medicine
Department of Medicine
The Bowman Gray School of Medicine
Wake Forest University
Winston-Salem, North Carolina*

INTRODUCTION

Medical oncology is the discipline that specializes in the use of systemic forms of treatment for the management of patients with cancer. This focus on *systemic* therapy distinguishes medical oncology from surgical and radiation oncology, which are best suited for treating localized cancers. In practice, the medical oncologist typically spends less time dealing with issues specifically related to the delivery of chemotherapy and more time confronting the fundamental issues of general oncology: establishment of a tissue diagnosis of malignancy; efficient staging of disease; review of prognosis and various treatment options with the patient and family; effective relief of pain; and coordination of various auxiliary services to ensure that the patient's emotional and social needs are met promptly.

Medical oncology originated in the 1940s when nitrogen mustard was first used to obtain a brief remission in a patient with lymphoma (Gilman 1963). Most of the specialty's major advances—notably in the treatment of childhood acute lymphocytic leukemia (ALL), choriocarcinoma, Hodgkin's disease (HD), testicular carcinoma, and diffuse large cell lymphoma—have followed the introduction and combination of other cytotoxic drugs.

Most cytotoxic drugs are effective in destroying cancer cells because they interfere directly or indirectly with the synthesis or function of nucleic acids. However, chemotherapeutic agents also produce undesirable damage to normal tissues, such as hematologic suppression, mucositis, and hair loss. Many patients also fear the use of these drugs because of their tendency to cause nausea and vomiting. Major advances in understanding the pharmacokinetics and pharmacodynamics of these agents have allowed for less toxicity to normal

tissues and have aided the management of chemotherapy-induced nausea and vomiting.

Medical oncology changes rapidly as new agents with entirely different mechanisms of action are introduced. These include interferons, interleukins, hematopoietic colony stimulating factors, growth factor antagonists, and agents capable of inducing cellular differentiation. Medical oncology has also begun to make significant contributions to the treatment of localized disease, as witnessed by the combination of 5-fluorouracil (5-FU) and radiation therapy in the curative therapy of rectal carcinoma—a tumor formerly curable only by radical surgical excision.

The kinetics of tumor growth are important in understanding how chemotherapy may thwart the growth of malignant neoplasms, and why it so often fails to do so. From a clinical standpoint it is most relevant to describe a tumor's growth as that of a whole tissue, but the concepts of tissue growth are built upon observations regarding the growth of individual cells.

CELL CYCLE KINETICS

The familiar model of normal and malignant cell proliferation is the result of studies combining tritiated thymidine ($^3\text{H-TdR}$) labeling of cells with autoradiography (Cleaver 1967). Thymidine, the nucleoside precursor of the DNA nucleotide deoxythymidine monophosphate, is essential for the synthesis of DNA; a cell replicating its genome in preparation for mitosis incorporates thymidine into its nucleus. In a typical experiment $^3\text{H-TdR}$ is injected into an animal or a patient and fresh tissue is obtained by biopsy at serial intervals thereafter. The tissue so exposed to the isotope is overlaid with a photographic emulsion; the low-energy

beta-particles emitted by the tritium have a very short range, so only the silver grains lying directly above labeled nuclei are activated.

Such experiments led to the critical observation that DNA synthesis is not a continuous process from one mitosis to the next, but rather one known as S-phase (Howard and Pel 1951) that occurs during a discrete period of the intermitotic time. The intermitotic time has been subdivided into five phases (fig. 5.1). G_1 , the time or "gap" between mitosis and S-phase, is the portion of the cell cycle dedicated to fulfilling the specialized functions of a given cell type. During G_1 , the cell's energy is directed toward the synthesis of RNA and protein designed to execute these functions. G_2 , the gap between the end of S-phase and mitosis, represents the usually brief time required to organize the nucleus for the events of mitosis. The onset of mitosis (M) is marked by chromosomal condensation and is followed by chromosomal segregation and cell division, yielding two daughter cells. The fifth phase of the cell cycle, G_0 , is often depicted as lying outside of the loop connecting one mitosis with the next. This is because cells in G_0 do not respond to the signals that normally prompt initiation of DNA synthesis, in contrast to cells in G_1 .

However, cells in G_0 are by no means dead: they continue to synthesize RNA and protein and so may carry out some of the differentiative functions of a particular cell type. They often act as a reserve population of cells that, given the appropriate cues (e.g., increased availability of nutrients), can re-enter the pool of proliferating cells and repopulate a tissue.

Measurement of the duration of the various cell cycle phases is conceptually simple. Because thymidine is either incorporated into DNA or rapidly metabolized and excreted, only those cells that are in S-phase at the time of $^3\text{H-TdR}$ administration will develop grains over their nuclei on an autoradiograph. A cohort of cells labeled in S-phase can be followed by obtaining serial biopsies of tissue for autoradiography following the $^3\text{H-TdR}$ injection, and by recording the percentage of mitoses that are labeled in each biopsy (Quastler and Sherman 1959). When the percentage of labeled mitoses (PLM) is plotted against the time following $^3\text{H-TdR}$ injection, a curve such as that in fig. 5.2 is obtained. Cells that are at the very end of S-phase during $^3\text{H-TdR}$ labeling will be the first to produce labeled mitoses; the time that elapses between their exposure to $^3\text{H-TdR}$ and the appearance of these first labeled mitotic cells is

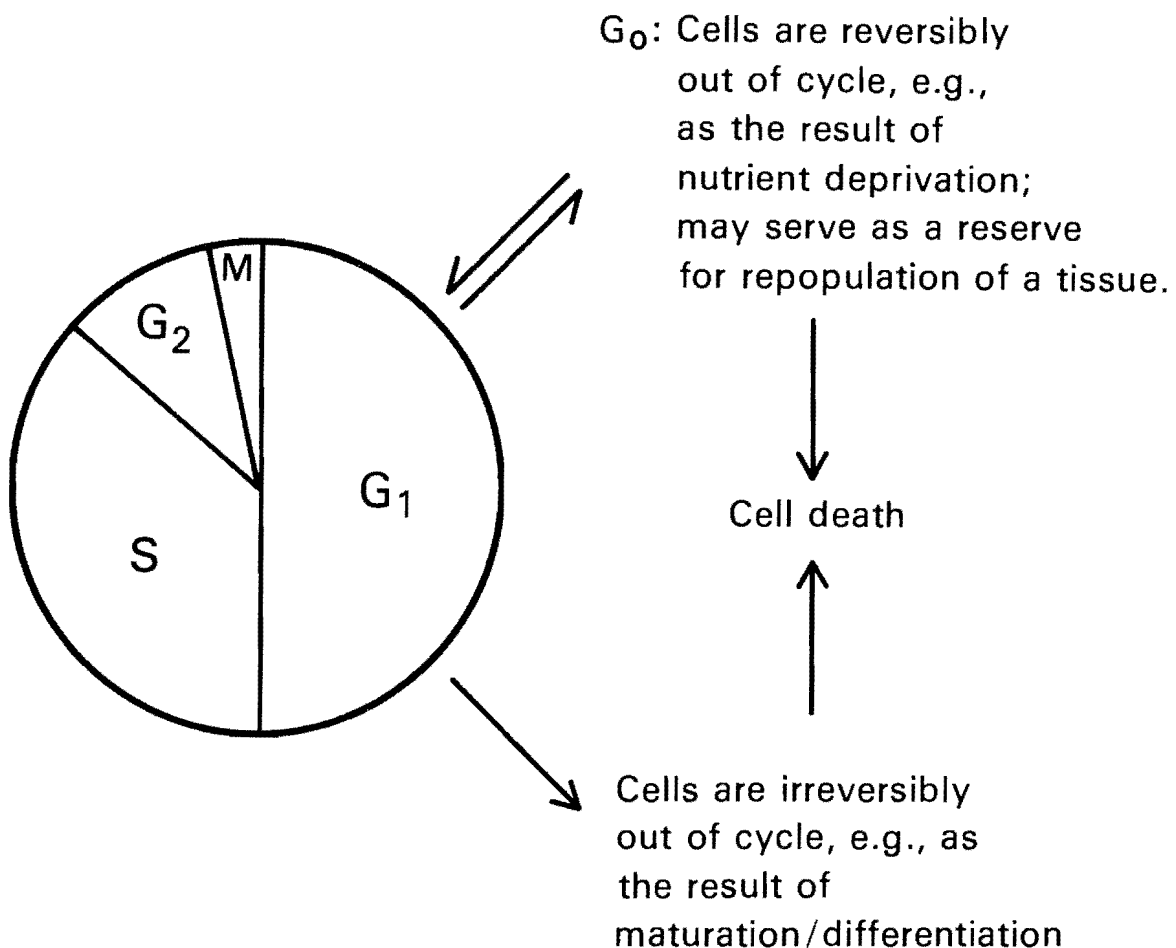


Fig. 5.1. Diagrammatic representation of the cell growth cycle, emphasizing the relationships between proliferating cell populations.

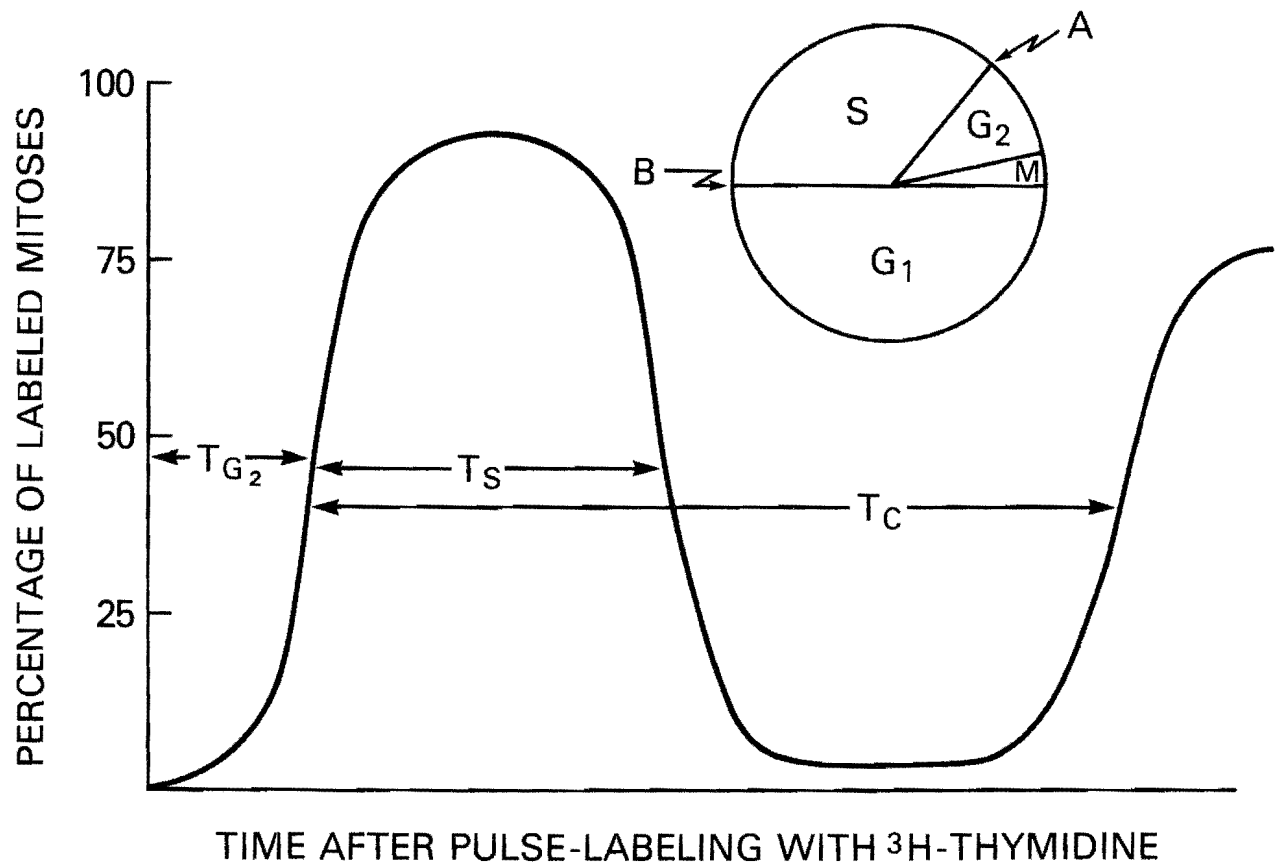


Fig. 5.2. Determination of cell cycle times using the percent labeled mitoses method. Only cells in S-phase become labeled at the time of ^3H -thymidine administration. Those cells located at the very end of S-phase (point A) are the first to appear in mitosis, and those just beginning to enter S-phase (point B) are the last to do so. The time lag between ^3H -thymidine pulsing and the appearance of the first labeled mitoses represents the time required for cells to pass through G_2 . The width of the waves of labeled mitoses gives the duration of S-phase, and the time separating one wave from the next yields the total cell cycle time.

the length of G_2 . Cells poised at the beginning of S-phase during labeling are the last to appear in mitosis, so the width of the wave reflects the duration of S-phase (T_S). If tissue samples continue to be examined at later points in time, then subsequent waves of labeled mitoses appear as the daughter cells of those originally labeled pass through mitosis. The time separating the beginning of one wave from the beginning of the next yields the total cell cycle time (T_C).

When the cell cycle kinetics of mammalian cells are examined *in vivo* using the PLM method, the durations of S, G_2 , and M phases are found to be remarkably similar—about seven hours, three hours, and one hour, respectively. In most instances there is no significant difference in the duration of these phases between normal and malignant tissues. The longest and most variable phase is G_1 , ranging from two to three hours to several days. Surprisingly, the intermitotic time (T_C) for most normal human cells is one to two days, while that of most malignant cells is approximately two to three days (Tannock 1978).

Cell cycle kinetics alone do not adequately describe tumor growth. First, in spite of similar values for T_C , different malignancies have distinctly different growth rates. For example, in acute myelogenous leukemia

(AML) T_C has been estimated at roughly 2.5 days, and in squamous cell carcinoma of the skin T_C has been measured at about 2.0 days (Tannock 1978). Yet AML left untreated results in death within weeks of diagnosis, while squamous cell carcinoma takes a more indolent course usually measured in years. Second, if tumor growth were solely determined by the cell cycle time, then tumors would be expected to double in volume every two to three days. Burkitt's lymphoma can come close to achieving this growth rate, but it is an exception rather than the rule. These incongruities can be resolved by describing tumor growth in the context of a whole tissue.

TISSUE GROWTH KINETICS

Tumors examined as whole tissues clearly show that not all of the cells are proliferating, that is, not all are "in cycle." Proliferating cells are a minority; the remaining bulk of the tumor's cells are in G_0 , are differentiated to the point that they no longer have the potential to replicate, or are dead. The fraction of tumor cells that *is* in cycle can be calculated through the use of ^3H -TdR autoradiography. The first step is to determine the labeling index (LI), the proportion of cells synthesizing

DNA at the time of exposure to the isotope (or, the proportion of cells that develops labeled nuclei). Then, if T_s and T_c are known for the cells of that tissue, as might be estimated by the PLM method, the fraction of proliferating cells in the tumor—called the growth fraction (GF)—is given by the following relation:

$$GF = LI \times T_c/T_s \times \lambda$$

The labeling index does not by itself give the fraction of proliferating cells, because not all cells that are in cycle will be in S-phase at the time of $^3\text{H-TdR}$ exposure; hence the term T_c/T_s to make the correction. The constant λ (always close to 1) is necessary to adjust for the fact that the distribution of cells in different phases of the cell cycle is not strictly equal (each cell going through mitosis yields two daughter cells, so the relative number of cells in each phase of the cell cycle decreases from G_1 to mitosis). The LI for a variety of solid tumors has generally been low, ranging from 1% to 8% (Tannock 1978); the LI of the epithelium of the normal gut is roughly 16%.

In addition to a low growth fraction, most tumors have a high rate of cell loss that further limits their growth rate (Steel 1967). A high percentage of new daughter cells die, in part due to the inherent genetic instability of malignant cells. Tumors also tend to outstrip their vascular supply and develop large areas of ischemic necrosis. Thus, most tumors are *not* what they appear to be on the surface—seething masses of rapidly dividing cells. Yet the nature of tumor growth is progressive, so clearly the rate of cell loss ultimately lags behind that of cell production. This imbalance between cell production and cell loss—the fact that tumors are not appropriately checked by the homeostatic mechanisms that maintain a predetermined number of cells in normal tissues—lies at the heart of tumor progression.

The significance of this imbalance is well-demonstrated by comparing acute with chronic myelogenous leukemia (Tannock 1978). Acute myelogenous leukemia (AML) is a rapidly progressive disease, killing patients within a matter of weeks to months if left untreated. On the other hand, chronic myelogenous leukemia (CML) usually follows an indolent course over several years. Surprisingly, the LI for myeloblasts in AML is about 5% to 11%, while that for the myeloblasts of CML may be as high as 43%. The difference is that the CML myeloblasts, in all but the terminal stages of the disease, are able to differentiate to more mature progeny (e.g., neutrophils) that have only a brief life span. The AML myeloblasts rarely produce more mature successors and, given their longer life span *vis-à-vis* mature cells, they accumulate rapidly. Even in the terminal phase of CML (“blast crisis”), when the percentage and number of myeloblasts increase rapidly, the LI does not change. The problem at this point in the illness is that the myeloblasts are no longer able to further differentiate and mature, and consequently the tempo of the disease becomes similar to that of AML.

GOMPERTZIAN GROWTH

The growth of a tumor changes drastically through time, a fact that has important implications for cancer treatment in general and for cancer chemotherapy in particular. During the early phases of a tumor's life span both the rate at which cells are produced and the rate at which cells are lost from the tumor are proportional to the number of cells in the tumor at a given time. Since cell number (N) and tumor volume (V) are proportional,

$$\frac{dv}{dt} = (K_p - K_L) \times V$$

where K_p equals the rate constant for cell production and K_L equals the rate constant for cell loss. Rearrangement and integration of the above relation gives the more useful relation

$$V_2 = V_1 e^{(K_p - K_L)(t_2 - t_1)}$$

In short, for a good portion of its life a tumor grows exponentially. This simple exponential relationship allows the doubling time of the tumor (T_D) to be calculated:

$$\frac{\ln 2}{K_p - K_L} = T_D$$

By measuring the time required for a tumor to double in size, it should be possible to estimate how long it took for the tumor to achieve a given size from its beginning as a single cell. Calculations made using tumor-doubling times obtained from measurements in patients would lead to the conclusion that the tumor must have started 10 to 20 years prior to its detection.

Such calculations are fundamentally flawed. With occasional exceptions, by the time a tumor becomes clinically detectable it has achieved a mass of approximately 1 g or 10^9 cells. To reach this size the tumor has already undergone about 30 doublings and its growth is no longer exponential. The additional 10 doublings required to produce 10^{12} cells or 1 kg—the tumor burden at which most patients die—occur much more slowly than the previous 30 doublings and represent a minority of the tumor's growth.

A German insurance actuary named Gompertz developed a mathematical model to describe the relationship between an individual's age and his expected time of death. The asymmetric sigmoidal curve resulting from this model comes close to describing accurately the growth of a tumor over its entire life span (see fig. 5.3). When the Gompertzian equation is used to calculate the time required for a tumor to reach 10^9 cells, it is estimated that most human malignancies originate less than two years prior to their clinical detection. The exact mechanisms influencing the shape of the Gompertzian curve are not well understood, but the slowing of growth in larger tumors is undoubtedly due in part to: hypoxia, as tumors outstrip their fragile vascular supply; decreased availability of nutrients and hormones; accumulation of toxic metabolites; and inhibitory cell-to-cell communication.

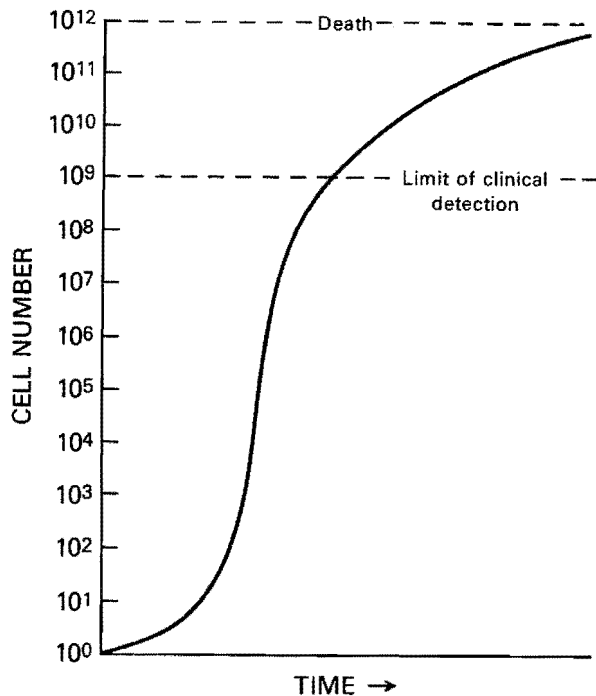


Fig. 5.3. The Gompertzian growth curve. During the early stages of its development a tumor's growth is exponential. But as a tumor enlarges, the growth slows. By the time a tumor becomes large enough to cause symptoms and be clinically detectable, the majority of its growth has already occurred and is no longer exponential.

Given that the majority of chemotherapeutic agents in common clinical use interfere directly with the synthesis or function of DNA, it is not surprising that these drugs are in general more toxic to proliferating cells than to those incapable of replication, or in G_0 , and are more effective against tumors with high-growth fractions (Shackney, McCormack, and Cuchural 1978). However, by the time tumors are clinically detectable they lie high on the Gompertzian curve, where their growth fractions are low. If the number of cells in a tumor is reduced by surgical debulking or radiation therapy, then the tumor has been brought to a lower point on the Gompertzian curve. Cells previously in G_0 re-enter the cell cycle, the tumor's growth fraction increases, and its growth rate may be similar to the rate of a tumor that had just attained the same size from a single cell. If effective chemotherapy is available, it would now likely be even more effective against the tumor. This concept of moving tumors down on the Gompertzian curve prior to the delivery of chemotherapy underlies much of the rationale behind adjuvant chemotherapy (Weiss and DeVita 1979), that is, chemotherapy given to patients who have no overt evidence of residual disease following local treatment (such as surgery or radiation therapy for a primary breast cancer), but for whom past experience indicates that similar patients have a high chance of relapse from the presence of undetectable micrometastatic disease.

THE STEM CELL MODEL OF TUMOR GROWTH

A further impediment to effective chemotherapy is the fact that proliferating cells, those that are most vulnerable to

the toxic effects of chemotherapy, are not necessarily the cells that must be eliminated in order to eradicate a tumor. Instead, the critical population of cells responsible for a tumor's persistence and growth is often largely in G_0 . The reasons for this apparent paradox are found in the stem cell model of tissue growth.

The stem cell model has been most extensively elaborated for the cells of the bone marrow, which are depicted as components of a complex hierarchy. At the top of the hierarchy are relatively undifferentiated cells which have an unlimited capacity for self-replication and for replenishing the marrow with all of its various elements, and yet which have virtually no ability to perform the ultimate functions of the marrow (oxygen transport, hemostasis, and defense against infection). At the bottom are those mature, highly specialized cells (neutrophils, erythrocytes, and platelets) that can execute these tasks but which have no ability to divide and renew themselves. Although the proliferative rates for the immediate descendants of the stem cells tend to be high—the LI index for myeloblasts and myelocytes is 40% and 20%, respectively, and that of early erythroid precursors ranges between 30% and 75% (Tannock 1978)—the stem cells tend to proliferate slowly unless provoked by an appropriate stimulus (e.g., blood loss or infection).

The sluggish proliferation of marrow stem cells has been inferred by observing the extent to which their numbers are reduced by the administration of very high doses of $^3\text{H-TdR}$ —a technique dubbed "thymidine suicide." As in $^3\text{H-TdR}$ autoradiography, only cells synthesizing DNA (those in S-phase) will accumulate the isotope in their nuclei. However, in the thymidine suicide experiments the doses of $^3\text{H-TdR}$ are high enough to be lethal.

The original stem cell assays of bone marrow were performed in mice (Till and McCullough 1980). A mouse given a sufficiently large dose of radiation will fail to recover hematopoietic function; both the mature cells and the stem cells of the marrow are irrevocably damaged. If, however, this lethally irradiated mouse is transfused with marrow from a nonirradiated syngeneic mouse, its marrow is repopulated and hematopoiesis resumes. The transfused marrow will also establish colonies of hematopoiesis in the spleen, each colony derived from a single stem cell. These spleen cell colonies contain granulocytes, erythrocytes, platelets, and their less mature precursors, so the founding stem cell is referred to as pluripotent, that is, capable of giving rise to cells of all three lineages. When normal bone marrow is exposed to high doses of $^3\text{H-TdR}$ and then transfused into a lethally irradiated, syngeneic mouse, the number of spleen colonies formed is reduced only slightly compared to the number obtained with transfusion of marrow not exposed to the isotope. The conclusion is that the marrow's stem cells are not rapidly proliferating; that is, most are in G_0 . Yet if the donor marrow is stimulated to proliferate, as might occur if the donor were rendered anemic by phlebotomy, then exposure of that marrow to $^3\text{H-TdR}$ before

transplantation results in a more pronounced reduction in the number of spleen colonies formed (Becker et al. 1965). The induction of anemia caused the stem cells to move out of G_0 and back into the cell cycle, so that the deficit in erythrocytes might be corrected.

The stem cell model of tissue growth has been extended to describe the growth of nonhematopoietic tissues, including tumors. Several lines of evidence argue in favor of using this model to describe malignant tissues. First, it is well established that nearly all tumors arise from a single, pluripotential, albeit aberrant founding (stem) cell. The possibility that most human tumors are monoclonal is strongly suggested by the unique paraprotein of multiple myeloma; by cytogenetic studies in leukemia (the best example of which is the 9:22 translocation in CML); and by the finding of a single G-6-PD isoenzyme in the tumor cells of women heterozygous at the G-6-PD locus.

Second, tumors, like the bone marrow, contain cells of varying degrees of differentiation. The more differentiated cells have low rates of proliferation and, when transplanted, are incapable of establishing new tumors; the less differentiated cells have higher proliferative rates (just as the LI for myeloblasts is higher than that for myelocytes) and are more efficient at founding new tumors. The more anaplastic tumors tend to have higher growth fractions and follow a more fulminant course if left without effective treatment.

The third point in favor of the stem cell model for tumor growth is the response of some human tumors to radiation therapy. Radiation therapy can cure tumors that arise spontaneously in humans at doses that would be incapable of curing experimental tumors of the same size in mice. The increased sensitivity of the spontaneous human tumors appears to reflect a smaller population of stem cells.

Direct measurement of stem cells in tumors has been accomplished through mouse-spleen colony assays (for example, injection of lymphoma cells into a mouse with the ensuing appearance of lymphoma colonies in the spleen); the enumeration of metastatic lung colonies following the intravenous injection of tumor cells into mice; and tumor colony formation *in vitro* (Hamburger and Salmon 1977). The last method has yielded mixed results in human tumors (Selby, Buick, and Tannock 1983). The impetus for developing of an *in vitro* assay of human tumor stem cells was the hope that it would allow for more rational drug therapy. By noting the effect of varying concentrations of different chemotherapeutic agents on stem cell colony formation, it might be possible to predict which drugs would be most likely to have activity *in vivo*. Unfortunately, such assays are good at predicting which drugs will not be effective, but they fail to predict accurately which drugs will succeed. Nonetheless, the stem cell assay demonstrates that only 1 in 1,000 to 1 in 10,000 cells in a tumor is capable of forming colonies of cancer cells *in vitro*. Although this poor cloning efficiency could be due in part to technical problems related to grow-

ing cells in culture, it is more likely a manifestation of the fact that most cells in a tumor have a very limited potential, if any, for self-renewal.

There is controversy over the proliferative status of stem cells in human tumors. Some studies indicate that they spend less time in G_0 than do their bone marrow counterparts (Shimizu et al. 1982; Minden, Till, and McCulloch, 1978). Nonetheless, the proliferating cells of a tumor—perhaps the cells that respond most dramatically to chemotherapy and yield clinical regression of disease (albeit transient)—are not necessarily the cells that need to be eradicated to effect a cure. Thus, the response of a tumor to a drug is best assessed by measuring the survival of clonogenic or stem cells, not of all tumor cells.

RELATIONSHIPS BETWEEN TUMOR CELL SURVIVAL AND DRUG DOSE

For most of the commonly employed chemotherapeutic agents, the relationship between tumor cell survival and drug dose is exponential, with the number of cells surviving a given dose of drug being proportional to both the dose of the drug and the number of cells at risk for exposure to the drug:

$$dN \propto NdD$$

where N = number of cells in the tumor
and D = dose of drug

or

$$dN = -KNdD$$

where the proportionality constant, $-K$, is introduced with a negative sign since the number of cells decreases with increasing drug dose. Rearranging and integrating the equation above yields the more useful formula:

$$N = N_0 e^{-K(D - D_0)}$$

where the subscript "0" indicates the initial dose and cell number.

A simple exponential relationship implies that the death of a tumor cell is the consequence of a simple interaction between the drug molecule and its target in the cell. Of more immediate clinical relevance, exponential cell killing implies two things: multiple courses of therapy will be needed to eradicate the tumor, since with each dose of drug the same proportion of cells—*not* the same number of cells—is killed; and small changes in the dose of a drug may translate into large changes in cell survival.

For example, assume that at the beginning of treatment a tumor contains 10^{10} cells. If each course of treatment results in the death of 99.9% of these cells, and if 1 log of cell growth occurs between courses of treatment, then five courses of treatment are required to eliminate the last cell (fig. 5.4) This reasoning assumes an ideal situation wherein all cells are equally sensitive to the drug, there are no cells resistant to the

drug present at the outset of therapy, and no cells become resistant during therapy. These assumptions are not valid during the treatment of spontaneously occurring human tumors (nonkinetic forms of drug resistance are the major impediment to the successful treatment of human tumors), but the example makes the point that repeated courses of treatment are necessary. It also demonstrates that complete clinical remission, typically achieved when the number of tumor cells falls below 10^9 , does not equal a cure; treatment must be continued despite the fact that no overt tumor is present. Continued treatment with potentially toxic drugs when no tumor is clinically evident is difficult for both the patient and the oncologist. Only rarely does the oncologist have the benefit of being able to follow a sensitive and specific tumor marker (e.g., β -HCG in the management of choriocarcinoma) to monitor the effect of treatment beyond the point of complete remission.

The disproportionate increase in cell survival that results from a decrease in drug dose is shown with a simple example. Assume that a tumor before any treatment contains 10^{11} cells. Also assume that the proportionality constant, $-K$, is -5 for the alkylating agent cyclophosphamide when used to treat the tumor. If a dose of 1.5 g of cyclophosphamide is delivered, then the tumor would be left with 5.5×10^7 cells:

$$N = N_0 e^{-K(D-D_0)}; N_0 = 10^{11} \text{ when } D_0 = 0$$

$$N = 10^{11} e^{-5(1.5-0)} = 5.5 \times 10^7 \text{ cells}$$

What happens if the oncologist chooses to administer 0.75 g of cyclophosphamide instead of 1.5 g?

$$N = 10^{11} e^{-5(0.75-0)} = 2.4 \times 10^9 \text{ cells}$$

The result is that a 50% decrease in dose has translated into a 98% increase in cell survival.

Reduction of the dose of a drug given to a patient is often unavoidable because of undue toxicity to normal tissues (e.g., myelosuppression, mucositis). Still, giving drugs in full doses (which implies both the amount and frequency with which a drug is given) is an important goal when treating patients. A retrospective analysis of women receiving adjuvant chemotherapy for stage II, node-positive carcinoma of the breast indicated that those women who received the full dose of scheduled drugs were less likely to develop recurrent disease than those who were given lesser doses (Hryniuk, Levine, and Levin 1986).

Plots of cell survival as a function of drug dose have rarely been constructed for human tumors under treatment *in vivo*. However, such curves have been constructed for animal tumors using the spleen-colony assay or a metastatic lung-nodule assay, and for both animal and human tumor cell lines cultured *in vitro*. These experimental systems measure the survival only of clonogenic tumor cells as a function of drug dose;

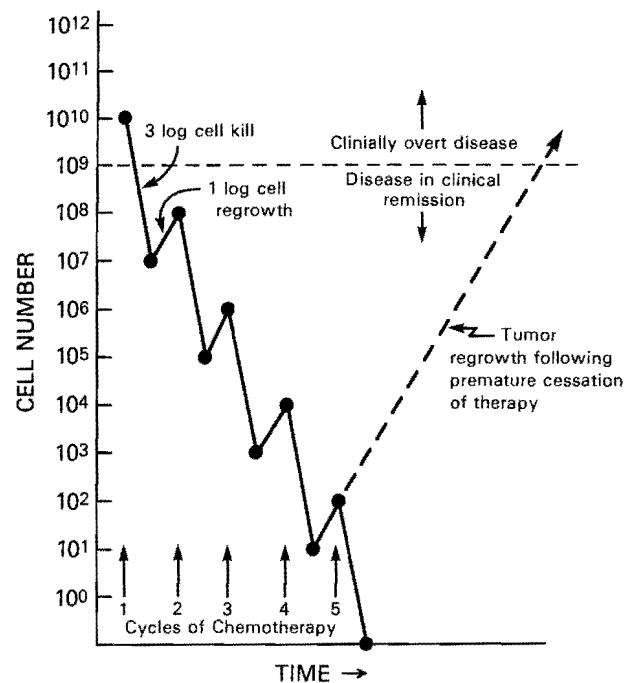


Fig. 5.4. Relationship between tumor cell survival and chemotherapy administration. The exponential relationship between chemotherapy drug dose and tumor cell survival dictates that a constant proportion, not number, of tumor cells is killed with each cycle of treatment. In this example each cycle of drug administration results in 99.9% (3 log) cell kill, and 1 log of cell regrowth occurs between cycles. The broken line indicates what would occur if the last cycle of therapy was omitted: despite complete clinical remission of disease, the tumor would ultimately recur.

clonogenic cells are the cells of interest inasmuch as it is their elimination that is necessary for a cure.

In the spleen-colony assay, tumor cells such as those from a murine lymphoma (Bruce et al. 1969) are injected into a lethally irradiated syngeneic mouse following exposure of the tumor cells to varying doses of drug; the number of tumor cell colonies that subsequently appear in the spleen reflects the number of clonogenic cells surviving drug exposure. Some tumors will form metastatic lung nodules following intravenous injection, and stem cell survival can be assayed in this manner as well. The first conclusion to be drawn from such studies and the analogous *in vitro* cell culture assays is that the slope of the cell survival vs. drug dose curve is much steeper for rapidly proliferating cells than for those that proliferate slowly; that is, tumors whose clonogenic cells are more often "in cycle" (not in G_0) are more sensitive to a given dose of drug (Van Patten, Lelieveld, and Kram-Idsenga 1972). Only a handful of drugs—specifically cisplatin, the nitrosoureas, nitrogen mustard, and bleomycin—show little increased toxicity for proliferating cells (Twentyman and Bleehen 1975).

The second conclusion to be drawn from these curves is that some chemotherapy drugs exhibit a plateau in cell killing as dose is further increased (fig. 5.5). Drugs behaving in this manner are those which have their exclusive or major effect during a single phase of the cell cycle, characteristically during S-phase. In order to

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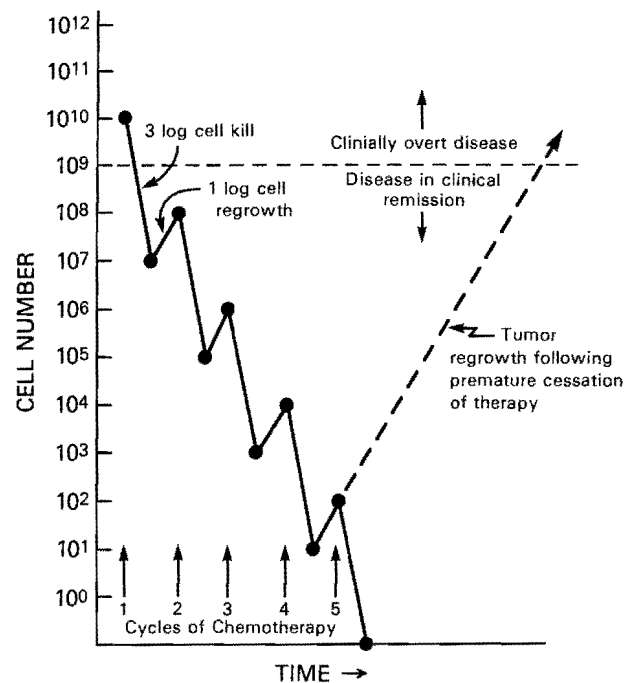


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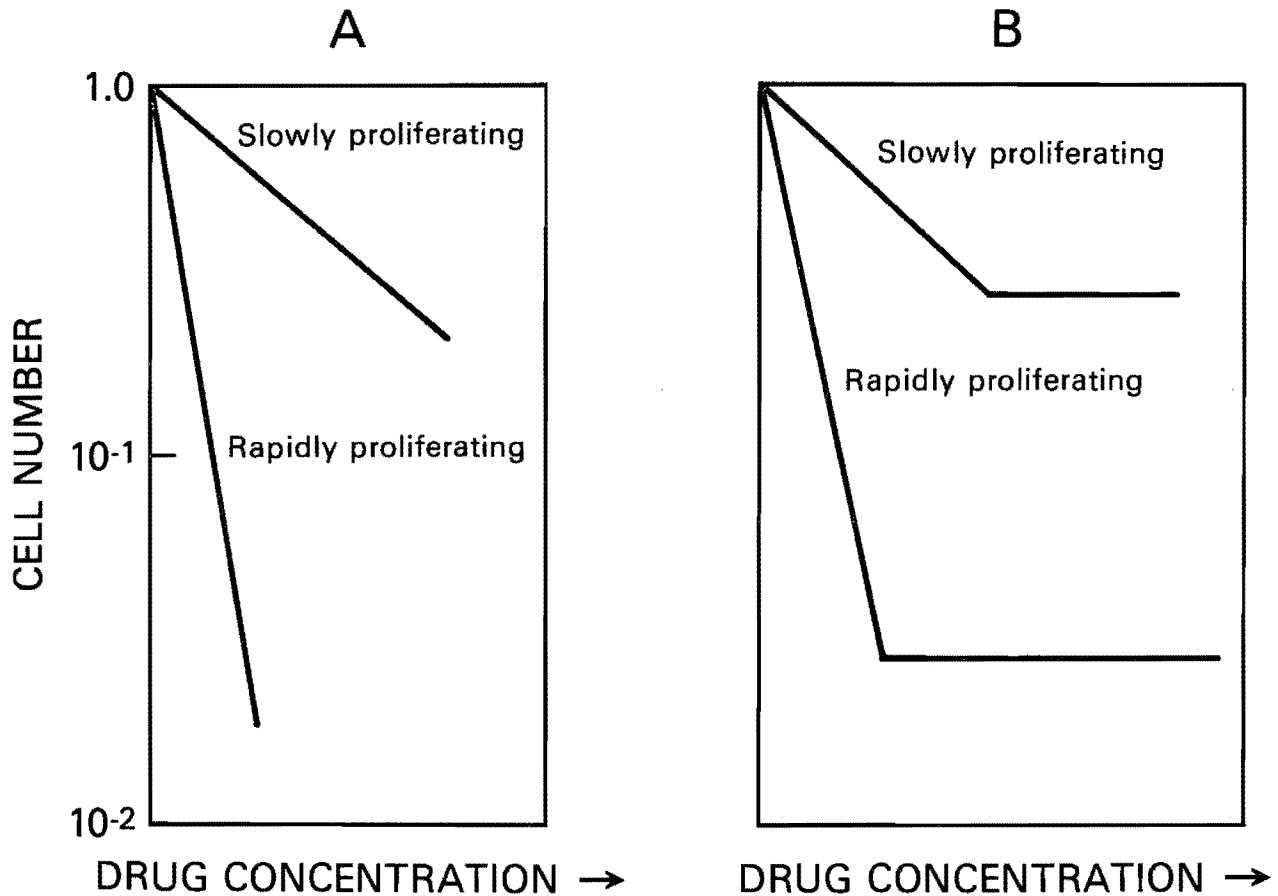


Fig. 5.5. Relationship between the proliferative rate of tumor cells and sensitivity to chemotherapy. (A) Cell populations are briefly exposed to varying concentrations of drug with subsequent determination of clonogenic cell survival. Rapidly proliferating cells, i.e., cell populations with few cells in G_0 , are more sensitive to a given dose of drug than are slowly proliferating cells. The drugs used in these experiments are without significant specificity for any one phase of cell cycle. (B) These experiments were performed as in A, but with drugs that are specific for one phase of cell cycle (e.g., S-phase). Again, rapidly proliferating cells are more sensitive to a given dose of drug than are slowly proliferating cells. However, there is now a plateau in survival with increasing drug dose for both types of cell populations. Further decrease in cell survival can be achieved only by prolonging the duration of exposure to drug, allowing more cells to enter the susceptible phase of cell cycle.

increase cell kill beyond the plateau level, the *duration* of exposure must be prolonged to allow more cells in the tumor to enter the susceptible phase of the cell cycle. The antimetabolites—drugs which are structurally similar to normal metabolites and which disrupt cell function by competing with these normal precursor molecules in critical metabolic pathways—are the most notable drugs of this type and include cytosine arabinoside (Ara-C), thioguanine, mercaptopurine, methotrexate, 5-fluorouracil (5-FU), and hydroxyurea. Recognition of the plateau in the cell survival vs. dose curve for antimetabolites has greatly influenced the manner in which several of them are administered to patients.

MECHANISMS OF DRUG RESISTANCE

Given that most chemotherapeutic agents are more toxic to rapidly proliferating tumors, and given that a tumor's proliferative rate increases as its size decreases, it should follow that a tumor, having responded favorably to an initial course of chemotherapy, would become increasingly sensitive to treatment during subsequent

courses of therapy. Unfortunately, this scenario rarely, if ever, transpires. More typically, tumors regress and may become clinically undetectable during the initial courses of chemotherapy, but later resurge with vigor despite continuation of the same therapy. Tumor growth kinetics clearly fail to explain this sequence of events.

Mechanisms other than a tumor's growth fraction or a cell's location in the cell cycle explain why malignancies are resistant to chemotherapy. First, tumor cells may reside in so-called sanctuaries inaccessible to most drugs. The central nervous system (CNS) and testes are often impervious to drugs that distribute freely to other tissues; these are frequent sites of relapse notwithstanding successful treatment elsewhere. A major advance in the treatment of childhood ALL came with the recognition that the meninges were often a site of recurrent disease and that prophylactic treatment of the CNS, either with radiation or intrathecal methotrexate, could increase the chances for cure in those children who had achieved a complete remission with systemic therapy. Second, drug resistance also may seem greater than it is, such as when a drug is incompletely absorbed, is rapidly

metabolized and excreted, or when it fails to be completely converted to its active form. In many treatment protocols these forms of pseudoresistance are at least partially circumvented by escalating drug doses until mild normal tissue toxicity occurs. By far the most important forms of drug resistance—the most typical explanations for treatment failure—lie in the genetic and biochemical makeup of malignant cells.

The genetic basis of drug resistance in malignant cells is now well established from several lines of evidence. The same procedures used to prove that bacterial drug resistance arises as a result of spontaneous mutation and subsequent selection (the fluctuation test of Luria and Delbruck) have been applied to tumor cells cultured *in vivo* (Goldie and Coldman 1984). If tumor cells are aliquoted in equal amounts onto media containing equivalent concentrations of a given chemotherapeutic agent, then the number of tumor colonies appearing on each of those media—the progeny of single resistant cells—is equal. The rate of development of drug resistance calculated from such experiments is consistent with known rates of spontaneous mutation—roughly one out of 10^6 to 10^7 cells. Moreover, if the tumor cells are initially plated onto media without drug and allowed to grow to a given size, and if they are thereafter subplated onto media with drug, then the number of colonies appearing in these subcultures varies significantly from one plate to the next. In the drug-free medium there is no selection pressure for drug-resistant cells, so their number varies widely, reflecting the random nature of spontaneous mutation. Other evidence includes the fact that the drug-resistant phenotype may persist despite the absence of drug, and the observation that mutagens may increase the rate with which drug-resistant cells appear. Perhaps most compelling are experiments in which DNA transfer from drug-resistant to drug-sensitive cells confers the drug-resistant phenotype upon the formerly vulnerable cells (Gros et al. 1986).

These arguments most readily apply to drug resistance arising from a point mutation, and on the surface they appear to exclude the idea that drugs may induce resistance to themselves by means other than simply increasing the rate of spontaneous mutation. However, a Lamarckian view of drug resistance has some validity.

If the only force causing tumor cells to become resistant were spontaneous mutation, then a tumor containing 10^9 cells, i.e., one which has just become clinically detectable, probably already contains 10^2 to 10^3 resistant cells (10^9 cells \times $1/10^7$ or $1/10^6$). If the rate of spontaneous mutation increases, then the smaller a tumor is, the less likely it is to contain drug-resistant cells. This conclusion makes intuitive sense, but what is not so readily apparent is the exponential relationship between tumor size and the probability of finding drug-resistant cells. Through the use of a mathematical model, Goldie and Coldman (1984) have predicted the following relationship:

$$P_0 = \frac{1}{e \alpha (N-1)}$$

where P_0 equals the probability of finding no drug-resistant cells within a tumor containing N cells and having a spontaneous rate of mutation α .

This relationship predicts that the curability of a tumor—here equated with the probability of there being no drug-resistant cells—does not decline gradually as the tumor grows larger, but instead decreases precipitously as the tumor approaches a critical size determined by its rate of spontaneous mutation. A tumor containing 10^6 cells and having a spontaneous mutation rate of 10^{-7} stands only a 10% chance of containing drug-resistant cells; but if the tumor is allowed to grow 1.5 logs (to 5×10^7 cells) this chance increases to more than 99%.

The clinical message behind the Goldie-Coldman model is that smaller tumors are easier to cure, and that the best moment for effective treatment is as soon after detection as possible. Delay in treatment, by allowing a modest increase in tumor burden, can result in a large increase in the probability of encountering drug-resistant cells and may so forfeit the patient's chance for cure or at least meaningful disease regression. An extension of the Goldie-Coldman model argues that patients should benefit from the use of multiple, noncross-resistant drugs (effectively decreasing the rate of spontaneous mutation), and also that equally effective, noncross-resistant regimens should be alternated, rather than given sequentially. The use of multiple chemotherapeutic agents in combination has been responsible for much of the success achieved in the treatment of childhood ALL, HD, and testicular carcinoma; single-agent therapy is clearly inferior for the treatment of these malignancies. The superiority of multiple drugs over single agents is not so clear in some of the more common solid tumors, such as colon cancer. The failure of multiple agents in the treatment of these tumors is not so much a failure of the Goldie-Coldman model, but rather a reflection of the inherently high levels of resistance these tumors have to all currently available chemotherapeutic agents. Also, there are practical limits on how far the theoretical advantages of combination chemotherapy can be taken. Two or even three drugs may produce activity against a given tumor, but if they all produce similar normal-tissue toxicities they will be difficult to combine in the clinic. Either the combination will result in excessive morbidity and mortality, or the drug doses will be reduced to levels where none is very effective.

Unfortunately, the problem of drug resistance is a complex one that goes well beyond the simple model of random spontaneous mutation to resistance and subsequent selection. The first level of complexity lies in the ability of some drugs to promote resistance against themselves (the Lamarckian view). Resistance to the antimetabolite methotrexate (MTX) can be effected through several mechanisms (Bertino et al. 1981). An

alteration in the cell surface membrane receptor for folates, as might occur by a point mutation, can reduce intracellular levels of MTX and result in less inhibition of the target enzyme dihydrofolate reductase (DHFR). Once MTX enters the cell a number of glutamic acid residues are added to the single glutamic acid moiety that forms one end of the MTX molecule; such polyglutamation, which also occurs with the normal substrate, folic acid, keeps MTX from exiting the cell and yet does not alter its ability to inhibit DHFR.

Decreased polyglutamation of the drug is another potential mechanism for resistance. Although not common, resistance to MTX may also be conferred by an alteration in the structure of the DHFR enzyme so that MTX binds to it less avidly. However, by far the most common form of MTX resistance (at least in experimental tumors and cell lines) is production by the resistant cell of exceedingly large amounts of DHFR through a process known as gene amplification (Schimke 1984). In this process a gene is replicated many times over while the cell is in S-phase. Such amplification is promoted by drugs like methotrexate, which transiently interrupt DNA synthesis in replicating cells. The degree to which the DHFR gene is amplified can be increased by gradually increasing the amount of methotrexate to which tumor cells are exposed. If cells so induced to amplify the DHFR gene are then withdrawn from MTX exposure they may lose some of their resistance to the drug; this phenomenon is related to the loss of amplified DHFR genes located in relatively unstable, poorly conserved extrachromosomal structures called double minutes.

MULTIDRUG RESISTANCE

A second level of complexity is that the development of resistance to one chemotherapeutic agent often results in the coincident development of resistance to other, albeit structurally unrelated ones (Biedler and Riehm 1970). The appearance of the multidrug-resistant phenotype is associated with the expression of a cell surface glycoprotein called P-170 (Juliano and Ling 1976). Cells expressing P-170 tend to manifest increased efflux of the drugs to which they are resistant; there is some evidence implicating P-170 as this so-called drug pump. The multidrug-resistant phenotype is stable even in the absence of exposure to the drugs to which resistance has developed.

Furthermore, the gene encoding the P-170 glycoprotein, called *mdr1*, has been isolated, sequenced, and transferred to a drug-sensitive cell line, so conferring resistance (Gros et al. 1986). Although the phenomenon of multidrug resistance was examined *in vitro*, it likely has relevance for the treatment of spontaneously arising tumors in humans. The P-170 glycoprotein has been identified in a resistant human ovarian cell line (Bell et al. 1985), and the *mdr1* gene has been expressed in some human tumors (Fojo et al. 1987). *In vitro* data indicate that compounds which inhibit calcium entry into cells (e.g., verapamil) interfere with calcium binding to calmodulin, and other drugs that stabilize cell membranes

(e.g., Quinidine) can partially restore drug sensitivity. The use of verapamil to circumvent P-170-associated multidrug resistance has produced initially encouraging results in patients with multiple myeloma and non-Hodgkin's lymphomas. In a small group of patients with these malignancies who had developed clinical resistance to treatment with a chemotherapeutic regimen consisting of vincristine, Adriamycin, and high-dose dexamethasone (VAD), the addition of verapamil to the regimen caused partial tumor regression in a subset (Dalton, Grogan, and Meltzer et al. 1989).

TUMOR CELL HETEROGENEITY

A third layer of drug-resistance complexity emanates from the genetic and phenotypic heterogeneity of human tumors (Schnipper 1986). Despite their common ancestry, it is the rule rather than the exception that tumor cells are diversified both genetically and phenotypically. Tumors, like their normal tissue counterparts, retain some of the originally programmed ability to mature and differentiate.

In the early stages of its growth, a tumor may differ phenotypically only slightly from its tissue of origin, but as it increases in size it characteristically becomes less differentiated or more anaplastic, and more difficult to treat. The genetic material of malignant cells is more unstable than that of normal cells, a fact most grossly manifested by multiple chromosomal defects (breaks, deletions, translocations, inversions, and the evanescent extrachromosomal double minutes) and expressed by higher mutation rates as measured by clonal selection for drug resistance. Chemotherapeutic agents may add to this genetic instability by acting as mutagens themselves. Hence, new subclones of malignant cells appear routinely; those with an increased capacity for self-renewal and proliferation gradually replace the more slowly growing, differentiated cells in the tumor, and drug-resistant clones replace sensitive ones in the face of systemic treatment. Classic examples of disease evolution include: Richter's syndrome, wherein the patient with CLL develops an aggressive diffuse large-cell lymphoma; and the progression of CML, initially an indolent myeloproliferative disorder with a single chromosomal abnormality (the 9;22 translocation), to a more fulminant disease (blast crisis), still accompanied by the 9;22 translocation but heralded by the accumulation of additional chromosomal abnormalities.

STANDARD CHEMOTHERAPEUTIC AGENTS

This chapter does not describe each of the standard chemotherapeutic agents in detail or give specific guidelines for their proper clinical use; this critical information is readily available from several sources (Dorr and Fritz 1980; Skeel 1987; Wittes 1989). Rather, the purpose is to introduce the major classes of cytotoxic drugs, emphasizing their mechanisms of action, pharmacology,