LIPPINCOTT MANUAL®

Manual of Clinical Oncology

8TH EDITION

Bartosz Chmielowski
Mary Territo

Wolters Kluwer

Manual of Clinical Oncology

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8TH EDITION

Editors

Bartosz Chmielowski, MD, PhD
Associate Clinical Professor
Jonsson Comprehensive Cancer Center
Division of Hematology and Oncology
University of California, Los Angeles
Los Angeles, California

Mary Territo, MD
Emeritus Professor of Medicine
Division of Hematology and Oncology
David Geffen School of Medicine
University of California, Los Angeles
Los Angeles, California

Philadelphia • Baltimore • New York • London
Buenos Aires • Hong Kong • Sydney • Tokyo

Executive Editor: Rebecca Gaertner
Acquisitions Editor: Ryan Shaw
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Marketing Manager: Rachel Mante Leung
Senior Production Project Manager: Alicia Jackson
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Contributors

Russell K. Brynes, MD
Professor of Clinical Pathology
Chief, Hematopathology Service
Department of Pathology
Keck School of Medicine
University of Southern California
Los Angeles, California

Pranatharthi H. Chandrasekar, MD, FACP, FIDSA
Professor, Internal Medicine and Infectious Diseases
Chief, Division of Infectious Diseases
Wayne State University
Chief, Infectious Diseases
Karmanos Cancer Institute
Detroit, Michigan

Howard A. Chansky, MD
Professor and Chair
Department of Orthopaedics and Sports Medicine
University of Washington School of Medicine
Seattle, Washington

Bartosz Chmielowski, MD, PhD
Associate Clinical Professor
Jonsson Comprehensive Cancer Center
Division of Hematology and Oncology
University of California, Los Angeles
Los Angeles, California

Darin J. Davidson, MD, MHSc, FRCSC
Assistant Professor
Musculoskeletal Oncology Service
Program Director, Musculoskeletal Oncology Fellowship
Department of Orthopaedics and Sports Medicine
University of Washington School of Medicine
Seattle, Washington

Lisa M. DeAngelis, MD
Professor of Neurology
Weill Cornell Medical College
Chair, Department of Neurology
Memorial Sloan Kettering Cancer Center
New York, New York

Chaitanya R. Divgi, MD
Professor of Radiology
Executive Vice-Chair, Research
Chief of Nuclear Medicine and Molecular Imaging
Department of Radiology
Columbia University
New York, New York

Alexandra Drakaki, MD, PhD
Assistant Professor of Medicine and Urology
Director—Genitourinary Medical Oncology Program
Division of Hematology and Oncology
Institute of Urologic Oncology
University of California, Los Angeles
Los Angeles, California

Martin J. Edelman, MD, FACP
Professor of Medicine
Head, Section of Solid Tumor Oncology
Associate Director, Division of Hematology and Oncology
University of Maryland Greenebaum Cancer Center

University of Maryland School of Medicine
Baltimore, Maryland

Lawrence H. Einhorn, MD
Distinguished Professor of Medicine
Department of Medicine—Hematology/Oncology
Indiana University
Indianapolis, Indiana

Herbert Eradat, MD, MS
Assistant Clinical Professor of Medicine
UCLA Lymphoma Program
Division of Hematology and Oncology
David Geffen School of Medicine at UCLA
Los Angeles, California

Robert A. Figlin, MD, FACP
Steven Spielberg Family Chair in Hematology Oncology
Professor of Medicine and Biomedical Sciences
Director, Division of Hematology and Oncology
Deputy Director
Samuel Oschin Comprehensive Cancer Institute
Cedars-Sinai Medical Center
Los Angeles, California

Charles A. Forscher, MD
Medical Director, Sarcoma Program
Samuel Oschin Cancer Center
Cedars-Sinai Medical Center
Assistant Clinical Professor
David Geffen School of Medicine at UCLA
Los Angeles, California

David R. Gandara, MD
Professor of Medicine

Director, Thoracic Oncology Program
Senior Advisor to the Director
UC Davis Comprehensive Cancer Center
Sacramento, California

Patricia A. Ganz, MD
Distinguished Professor Health Policy and Management and Medicine
UCLA Fielding School of Public Health
David Geffen School of Medicine at UCLA
Director, Cancer Prevention and Control Research
Jonsson Comprehensive Cancer Center
Los Angeles, California

Axel Grothey, MD
Professor of Oncology
Department of Oncology
Mayo Clinic College of Medicine
Rochester, Minnesota

Shireen N. Heidari, MD
Fellow in Palliative Medicine
UCLA Department of Medicine, Palliative Care Service
Los Angeles, California

Siwen Hu-Lieskovan, MD, PhD
Assistant Clinical Professor
Division of Hematology and Oncology
Department of Medicine
David Geffen School of Medicine at UCLA
Los Angeles, California

Carole G. H. Hurvitz, MD
Emeritus Director Pediatric Hematology Oncology
Cedars Sinai Medical Center
Samuel Oschin Comprehensive Cancer Institute

Emeritus Professor of Pediatrics
David Geffen School of Medicine
University of California, Los Angeles
Los Angeles, California

Amy A. Jacobson, RN, NP-BC
Clinical Research Nurse, Nurse Practitioner
UCLA-LIVESTRONG™ Survivorship Center of Excellence
Jonsson Comprehensive Cancer Center
Los Angeles, California

Pashtoon Murtaza Kasi, MD
Assistant Professor
College of Medicine/Oncology
Mayo Clinic, Florida
Jacksonville, Florida

Hyung L. Kim, MD
Medallion Chair in Urology
Director, Academic Programs, Urology
Co-director, Urologic Oncology
Associate Director, Samuel Oschin Comprehensive Cancer Center
Cedars Sinai Medical Center
Los Angeles, California

Mira Kistler, MD
Fellow in Hematology Oncology
Division of Hematology and Oncology
David Geffen School of Medicine at UCLA
Los Angeles, California

Rekha A. Kumbla, MD
Division of Hematology and Oncology
Olive View–UCLA Medical Center and Cedars Sinai Medical Center
Los Angeles, California

Sarah M. Larson, MD

Assistant Professor
Division of Hematology and Oncology
Department of Medicine
University of California, Los Angeles
Los Angeles, California

Steve P. Lee, MD, PhD

Professor of Clinical Radiation Oncology
Department of Radiation Oncology
David Geffen School of Medicine at UCLA
Los Angeles, California

Margaret I. Liang, MD

Gynecologic Oncology Fellow
Division of Gynecologic Oncology
Department of Obstetrics and Gynecology
University of California, Los Angeles
Los Angeles, California

Sandy T. Liu, MD

Hematology/Oncology Fellow
Division of Hematology and Oncology
David Geffen School of Medicine at UCLA
Los Angeles, California

Alison W. Loren, MD, MS, FACP

Director, Hematology/Oncology Fellowship
Associate Professor of Medicine
Division of Hematology and Oncology
Perelman School of Medicine of the University of Pennsylvania
Perelman Center for Advanced Medicine
Philadelphia, Pennsylvania

Sonia Mahajan, MD

Visiting Scientist
Department of Radiology
Columbia University
New York, New York

Carolyn Maxwell, MD
Assistant Professor of Medicine
Stony Brook University School of Medicine
Division of Endocrinology
Department of Medicine
Stony Brook Medicine
Stony Brook, New York

Sanaz Memarzadeh, MD, PhD
Professor
UCLA Division of Gynecologic Oncology
Department of Obstetrics and Gynecology
Department of Biological Chemistry
David Geffen School of Medicine
Los Angeles, California

Ronald T. Mitsuyasu, MD
Professor of Medicine
Director, UCLA Center for Clinical AIDS Research and Education
David Geffen School of Medicine at UCLA
University of California, Los Angeles
Los Angeles, California

Theodore B. Moore, MD, MS
Professor and Chief
Division of Pediatric Hematology/Oncology
Department of Pediatrics
David Geffen School of Medicine at UCLA
Los Angeles, California

Bhagyashri Navalkele, MBBS, MD
Infectious Diseases Fellow
Division of Infectious Diseases
Department of Internal Medicine
Wayne State University School of Medicine
Detroit, Michigan

Andrew Huy Cao Nguyen, MD
Clinical Research Assistant
UCLA Department of Family Medicine
University of California, Los Angeles
Los Angeles, California

Ronald L. Paquette, MD
Blood and Marrow Transplant ProgramCedars-Sinai Medical CenterSamuel Oschin Comprehensive Cancer InstituteLos Angeles, California

Mark D. Pegram, MD
Director of Breast Oncology Program
Medical Oncology
Stanford University
Stanford, California

Lauren C. Pinter-Brown, MD, FACP
Health Sciences Professor of Medicine
Chao Family Comprehensive Cancer Center
University of California, Irvine
Irvine, California

Antoni Ribas, MD, PhD
Professor of Medicine
Professor of Surgery
Professor of Molecular and Medical Pharmacology
Director, Tumor Immunology Program, Jonsson Comprehensive Cancer

Center (JCCC)
David Geffen School of Medicine
Chair, Melanoma Committee at SWOG
University of California, Los Angeles (UCLA)
Los Angeles, California

Kathryn J. Ruddy, MD, MPH
Associate Professor of Oncology
Mayo Clinic
Rochester, Minnesota

Jordan E. Rullo, PhD, ABPP
Assistant Professor
Board Certified Clinical Health Psychologist
Department of Psychology and Psychiatry and Division of General Internal Medicine
Mayo Clinic
Rochester, Minnesota

Gary Schiller, MD
Professor of Medicine
Director, Aramont Program for Clinical Translational Research in Human Malignancies
Hematological Malignancy/Stem Cell Transplantation Unit
David Geffen School of Medicine at UCLA
Los Angeles, California

Mary E. Sehl, MD, PhD
Assistant Professor
Division of Hematology and Oncology
Department of Medicine
Department of Biomathematics
David Geffen School of Medicine
University of California, Los Angeles
Los Angeles, California

Maie A. St. John, MD, PhD

Associate Professor-in-Residence
Samuel and Della Pearlman Chair in Head and Neck Surgery
Co-Director, UCLA Head and Neck Cancer Program
Department of Head and Neck Surgery
University of California, Los Angeles
Los Angeles, California

Mary Territo, MD

Emeritus Professor of Medicine
Division of Hematology and Oncology
David Geffen School of Medicine
University of California, Los Angeles
Los Angeles, California

Yoshie Umemura, MD

Fellow
Department of Neurology
Memorial Sloan Kettering Cancer Center
New York, New York

Maria E.Vergara-Lluri , MD

Assistant Professor of Clinical Pathology
Hematopathology Service
Department of Pathology
Keck School of Medicine
University of Southern California
Los Angeles, California

Richard F. Wagner, Jr., MD

E.B. Smith Professor of Dermatology
Director of Mohs Surgery and Cutaneous Oncology
The University of Texas Medical Branch
Galveston, Texas

David Wallenstein, MD
UCLA Department of Family Medicine
UCLA Family Medicine Ambulatory Palliative Care and Pain Clinic
UCLA Palliative Care Service
Medical Director
Skirball Hospice Program, Los Angeles Jewish Home
Los Angeles, California

Deborah J. Wong, MD, PhD
Assistant Clinical Professor of Medicine
Division of Hematology and Oncology
Department of Medicine
David Geffen School of Medicine
University of California, Los Angeles
Los Angeles, California

Rodolfo Zamora, MD
Acting Instructor
Department of Orthopaedics and Sports Medicine
University of Washington School of Medicine
Fellow, Orthopaedic Oncology
University of Washington
Seattle, Washington

In Memoriam
Dennis Casciato, MD
May 7, 1939–December 6, 2013

This edition of the *Manual of Clinical Oncology* is being dedicated to Dr. Dennis Casciato who passed away in 2013.

Dennis received his bachelor degree in Biophysics from UC Berkley in 1960 and his MD in 1964 from UC San Francisco. He began his training in Internal Medicine at Orange County Medical Center (1964–1966). He then

served as a Major in the US Army from 1966–1969. Following his tour in the Army, he returned to complete his residency and undertake his Hematology Fellowship at UCLA–Wadsworth Veterans Association Medical Center (1969–1971). He then continued at the Wadsworth VA hospital where he was a research associate in Hematology and Infectious Diseases (1972–1974), Chief of Postgraduate Training from 1974–1977 and Chief of Hematology from 1978–1983. He was a member of the UCLA School of Medicine faculty from 1973–2012. In 1983, he transitioned into private practice and continued as Chief of Medicine at hospitals in the San Fernando Valley in California where he lived with his wife, Joy, and raised his sons, Frank and Andrew.

The *Manual of Clinical Oncology* was a labor of love for Dr. Casciato, and through the years, he was devoted to maintaining the *Manual of Clinical Oncology* as a quality resource for the oncologic community. Dennis was a consummate clinician/teacher and a major champion for the importance of the humanistic aspects of medicine.

Preface

The *Manual of Bedside Oncology* was first published in 1983 as a concise guide to the bedside management of cancer patients. It was collaboration between Drs. Dennis Casciato and Barry Lowitz who were the primary authors of all of the chapters. Because of its popularity, a second edition was produced in 1988 with a change of name to *Manual of Clinical Oncology* in order to more accurately reflect the content. Drs. Casciato and Lowitz continued to collaborate and edit editions 3 and 4, and with the increasing complexity of oncology, began inviting experts in various areas to help with the writing of certain chapters. Dr. Casciato was the sole editor of the fifth edition and then invited Dr. Mary Territo to serve as associate editor on editions 6 and 7 in the area of hematologic malignancies. Dr. Casciato began organizing the eighth edition, but when he realized he had a serious illness and would not be able to proceed, he recruited Dr. Bartosz Chmielowski to serve as coeditor with Dr. Territo. Dr. Chmielowski is on the faculty of the UCLA School of Medicine and had contributed to editions 6 and 7 with his expertise in melanoma and sarcomas and has a well-known broad-based knowledge and experience in solid tumors.

Over the years, the Manual has grown to encompass the developments and advances in oncology that have occurred. For this eighth edition, we have tried to incorporate the major achievements in immunotherapies, biologics, and targeted therapies that have been developed. At the same time, we strive to continue the main goals that Dr. Casciato has had for the Manual since its inception, to present a comprehensive, concise, and current reference for the treatment of cancer patients and to reaffirm the unique relationship between the cancer patients and their doctors.

Bartosz Chmielowski
Mary Territo

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I General Aspects

1 Biology of Cancer and Implications for Clinical Oncology

Bartosz Chmielowski

I. HALLMARKS OF CANCER

All cancers originate from normal cells of the host. Hence, a normal cell must undergo a series of changes in order to become tumorigenic and finally malignant. The tumor is not only composed of malignant cells but also contains a number of normal cells that were recruited; they are required for tumor growth. The understanding of cancer biology must not be limited only to studying malignant cells but also must include analysis of its environment.

Several distinct capabilities have been described that characterize the processes of achieving the growth advantage by cancerous cell and of tumorigenesis.

A. Sustaining proliferative signaling. The growth of normal cells is in homeostasis, assuring the maintenance of the integrity of organs. Malignant cells have an ability to proliferate uncontrollably. Cancers use multiple mechanisms to sustain their proliferation:

1. Tumors may produce growth factors for which they have receptors in an autocrine fashion.
2. Tumors may stimulate surrounding normal tissues and these normal tissues provide growth factors for the tumor.
3. Tumor may become hypersensitive to growth factors through up-regulation of growth receptors or alterations of the structure of these receptors.

4. Finally, they may become independent of growth factors by the presence of somatic mutations activating downstream pathways, for example, BRAF mutations activating the mitogen-activated protein kinase (MAPK) pathway, or mutations in phosphoinositide-3-kinase (PI3K) leading to activation of the PI3K/Akt/mTOR pathway, or by altering the negative feedback loops.
5. Tumors may stimulate surrounding normal tissues, and these normal tissues provide growth factors for growth.

B. Evading growth suppressors. Normal cells use multiple mechanisms to regulate negatively cell proliferation; most of these processes occur through the products of activation of tumor suppressor genes. Loss of function of these gene products allows tumor cells to evade the inhibitory mechanisms. Multiple genes have been implicated, but most of their products act as a part of the network of processes, and fortunately the cells can frequently compensate for the loss of function of a single gene; that is, loss of the function of a single tumor suppressor is not sufficient to induce oncogenesis.

1. The **RB** protein is responsible for the control of the cell cycle and the switch from resting state to cell division, mainly in response to the stimuli outside of the cell. The cells that lack the RB protein do not have this control mechanism. The retinoblastoma gene (RB1) was the first of these abnormal genes to be discovered. Subsequently, a number of other suppressor gene abnormalities have been found, particularly in uncommon or rare hereditary diseases. Examples include Wilms tumor (WT1), familial polyposis (APC), familial melanoma (CDKN20), and familial breast and ovarian cancers (BRCA-1 and BRCA-2).
2. The **p53** protein (TP53) is also a cell cycle control protein, but it responds mainly to the intracellular stressors, and it can stop cell cycle until the abnormal processes have been corrected. It can detect DNA abnormalities, such as nucleotide mismatches and DNA strand breaks, including those caused by radiation and chemotherapy. The function of p53 is thought to be critical in preserving the integrity of the cellular genome.
   - **a.** When DNA lesions are detected, the p53 protein arrests cells in the quiescent G_1 and G_2 phases of the cell cycle, preventing cells from entering the DNA synthetic (S) phase of the cell cycle. The p53 protein can then induce repair mechanism proteins or trigger proteins, which cause apoptosis.

b. In the absence of intact apoptosis, cancer cells can continue through sequential cell divisions and accumulate nucleotide mismatches and progressive DNA mutations.

c. *In vitro* studies have shown that chemotherapy and radiation kill cancer cells through DNA damage, which triggers p53 protein–induced apoptosis. In contrast, p53 protein–deficient mouse thymocytes and resting lymphocytes remain viable after irradiation.

d. Many human cancers are found to have mutant p53 suppressor genes. Mutant p53 is characteristic of Li-Fraumeni syndrome, a hereditary autosomal dominant syndrome of both soft tissue and epithelial cancers at multiple sites starting at an early age.

3. The ***NF2*** gene and its product **Merlin**. Merlin is responsible for maintaining of contact inhibition through E-cadherins. Normal cells stop proliferating when a desired density of cells is achieved. This process is dysregulated in cancer (loss of contact inhibition).

4. The **LKB1** epithelial polarity protein is responsible for maintaining tissue integrity and contributes to the contact inhibition phenomenon. The functional LKB1 can even overcome signals originating from strong oncogenes such as *Myc*.

5. Tumor growth factor-β (**TGF-β**) functions as a suppressor of tumor growth, but in the advanced malignancies its function may change and it may lead to increased aggressiveness of cancer through promotion of the epithelial-to-mesenchymal transformation.

C. Resisting cell death.

1. Apoptosis. Under normal conditions, cells that get damaged undergo programmed cell death in the process of apoptosis. Cancer cells have ability to avoid this process despite apoptosis-inducing stressors such as DNA damage or oncogene hypersignaling. Apoptosis is balanced by activity of antiapoptotic molecules from the Bcl-2 family (Bcl-2, Bcl-x_L, Bcl-w, Mcl-1, A1) and of proapoptotic molecules Bax and Bak. Cancer cells may express higher levels of the antiapoptotic or lower levels of proapoptotic molecules. TP53 is able to induce apoptosis; cancer cells that lack this oncogene can escape it.

a. Apoptosis occurs in normal tissue reabsorption. Apoptosis also results in the disappearance during embryogenesis of webs between fingers of primates, allowing the formation of individual

digits. Apoptosis results in the elimination of normal senescent cells when they become old and useless and of thymic T cells that recognize "self" and thereby prevent immune attack by these cells on the host.

b. **Apoptosis eliminates cells with abnormal DNA** caused either by irreparable DNA damage or by inaccurate, incomplete, or redundant transcription of DNA. This is a major mechanism for maintaining chromosome number in cells of a particular species and in preventing aneuploidy. The process ensures that only cells that have fully and accurately replicated their entire DNA can enter mitosis.

c. **Apoptotic cells can be recognized microscopically.** Apoptotic cells show clumps of intracellular organelles in the absence of necrosis. The nuclei are condensed and fragmented; intracellular structures are degenerated and compartmentalized. As the cell falls apart, phagocytes take up the fragments. Unlike the process of cell necrosis, apoptosis does not cause an inflammatory response. Apoptosis requires synthesis of specific proteins that have been highly conserved throughout evolution.

d. **Caspases.** The final stage of the various death pathways is mediated through activation of the caspases, which represent a family of cysteine proteases. The activation of caspases is determined by the intrinsic and extrinsic pathways of apoptosis. The intrinsic pathway is a mitochondrial-dependent pathway mediated by the *Bcl-2* family of proteins. Exposure to cytotoxic stress results in disruption of the mitochondrial membrane, which then leads to release of protease activators. Caspase-9 is subsequently activated, setting off a cascade of events that commit the cell to undergo apoptosis. The extrinsic pathway is mediated by ligand binding to the tumor necrosis factor (TNF) family of receptors, which includes TNF-related apoptosis-inducing ligand and others, and certain essential adaptor proteins. These adaptor proteins recruit various proteases that cleave the N-terminal domain of caspase-8, which leads to activation of the caspase cascade.

2. **Autophagy** is a natural process that allows cells to break down intracellular organelles upon exposure to stressors with help of lysosomes and recycle nutrients. It is also a protective process in case of neoplastic transformation. This process is regulated by PI3-

kinase, AKT, and mTOR pathway and by protein Beclin-1. Cancer cells may use autophagy to recycle their nutrients and escape damaging agents.

3. **Necrosis** is another process of cell death in which cells increase in size and break, releasing multiple proinflammatory cytokines. Immune cells are attracted to the areas of necrosis to eliminate the remnants of cells. Cancer can use this process to induce an inflammatory proneoplastic environment, stimulate angiogenesis, and even use these cytokines to stimulate its own growth.

D. **Enabling replicative immortality.** Normal cells are able to undergo only a limited number of divisions before they undergo one of two processes: **senescence** (the cells remain dormant, nondividing, but their state can be possibly reversed) or **crisis/apoptosis** (irreversible process leading to cell death). This process is governed by the presence of telomeres on the ends of chromosomes. In physiologic conditions, telomeres shorten with each division and hence make cells more susceptible to apoptosis/senescence. Shortening of telomeres is also a natural defense mechanism against development of cancer: potentially neoplastic cells divide and lose parts of telomeres with every division until their DNA is unprotected and they enter into the state of crisis. The cells that are able to maintain the activity of telomerase, an enzyme that is responsible for lengthening of telomeres, can potentially proliferate uncontrollably and turn into cancer. In some cases, precancerous cells have actually a low level of telomerase and become more vulnerable to apoptosis, but at the same time, in the presence of other prooncogenic events, their DNA is more susceptible to breakage and formation of multiple new fusion products, which will eventually give growth advantage to these cells and assure neoplastic transformation.

E. **Inducing angiogenesis.** The growth of cells can occur only when required nutrients are delivered and waste metabolites are removed from the cell environment. This process requires the presence of blood vessels. It is possible that microscopic lesions rely on osmosis for delivery of nutrients, but it has been shown that early in carcinogenesis, the “angiogenic switch” occurs that promotes new blood vessel formation. Vascular endothelial growth factor-A (VEGF-A) is the main protein responsible for neoangiogenesis. Its action is counterbalanced by the activity of thrombospondin-1 (TSP-1). Interestingly, tumor vasculature is not normal appearing; blood vessels are distorted, branched, and leaky and areas of hemorrhage are

observed. Different cancers rely on neovascularization to a different degree; for example, renal cell carcinoma is highly vascularized, but pancreatic adenocarcinoma may have a paucity of new blood vessels. Not only cancer cells but also the cells of tumor environment such as infiltrating immune cells (macrophages, mast cells, neutrophils, myeloid progenitors) and bone marrow–derived vascular progenitor cells induce angiogenesis.

1. VEGF induces receptors for itself on mature and nonproliferating blood vessel endothelial cells. These normal, resting endothelial cells do not have the receptor until they are exposed to VEGF.

2. VEGF induces the production and activity of multiple other growth factors that contribute to blood vessel formation.

3. VEGF can be induced by *c-ras* and by other oncogenes and growth factors, which then induce further production of VEGF.

4. VEGF appears to prevent apoptosis in induced endothelial cells.

F. Activating invasion and metastasis. Cancer cells are characterized by a unique ability of local invasion and formation of distant metastasis. E-cadherin is one of most important cell surface adhesion molecules responsible for maintaining tissue integrity. Multiple cancers express E-cadherin at a low level and express molecules implicated in cell migration such as N-cadherin at higher levels.

Formation of distant metastasis is a multistep process and it consists of the following:

1. Local invasion

2. Migration into lymphatic and blood vessels

3. Spread of cancer cells through vasculature

4. Extravasation of cancer cells into tissue of remote organs

5. Growth of cancer cells in the new environment to form macroscopic tumors

Molecular events occurring during the process of formation metastasis resemble steps of embryonic morphogenesis. The process by itself has been named **epithelial–mesenchymal transition** (EMT) and involves genes playing a physiologic role in embryogenesis such as *Snail*, *Slug*, *Twist*, and *Zeb1/2*. These genes are regulated by the intracellular oncogenic events, but they can be also influenced by microenvironmental stimuli. Invasive and metastatic potential of cancer is strongly dependent on the cross talk between neoplastic cells and the stroma.

Some cancers are characterized more by the ability of local invasion rather than formation of distant metastasis. They can invade into the adjacent tissue (“collective invasion”), which is frequently seen in squamous cell carcinomas of the head and neck area, and cause significant morbidity. Other cancers can spread through spaces in the extracellular matrix (“amoeboid invasion”).

Tissue invasion and formation of distant metastasis are two different processes. When macroscopic metastases are seen, it implicates that cancer cells were able to adapt to the new tissue environment that is very different from the environment of the primary site. Most probably, metastasis uses the same hallmarks for its growth and survival as the primary site. It is a random process, and therefore we frequently see that metastases may be diagnosed many years after the treatment of the primary tumor. The difficulty with adaptation to the new tissue environment also explains why not all patients with circulating tumor cells end up with metastatic disease.

G. Reprogramming energy metabolism. In normal conditions, the cell metabolism depends mainly on aerobic glycolysis, in which glucose is metabolized into pyruvate in the cytoplasm and finally into carbon dioxide in the mitochondria. Cancer cells switch their metabolism from aerobic to anaerobic glycolysis, which is a less efficient way of producing of ATP. Cancer cells compensate by an increased use of glucose to achieve the same level of ATP. It is frequently achieved by up-regulation of glucose transporters such as GLUT1. Hypoxic conditions in the tumor environment further accentuate the process. Glycolytic intermediate processes are used by cancer cells for generation of nucleosides and amino acids that are essential for tumor proliferation and growth. In addition, some cancers have actually a mixed population of cells: some rely on glucose-dependent metabolism and some use lactate. Lactate is produced during anaerobic glycolysis and it can provide the fuel for neighboring cells. Gliomas and other cancers were found to contain activating mutations in the isocitrate dehydrogenase 1/2 (IDH) gene; the clones that contain these mutations have a growth advantage through altered metabolism.

H. Evading immune destruction. The function of the immune system in the control of growth of abnormal cells is insurmountable. The immune cells continue surveying the body and eliminate pathogens and potentially cancerous cells. For cancer cells to survive, they have to evade this surveillance and they can achieve it by disabling

components of the immune system. The detailed discussion on the tumor–immune system interactions can be found in Chapter 5.

II. ENABLING CANCER CHARACTERISTICS

Hallmarks of cancer are a set of features that allow malignant cells to proliferate, survive, and metastasize. These abilities are acquired through the development of various enabling characteristics.

A. Genomic instability and mutation. The process of progression from a normal cell to a premalignant cell and finally to a malignant cell is achieved because of the instability of the genome: proliferating cells generate new mutations in a stochastic way and only the clones that acquired features allowing them to evade the defense mechanisms can survive. This observation implies that premalignant cells would contain fewer mutations than metastatic lesions, and this has been shown to be true in most of cancers. Not all changes must be elicited by mutational changes; the epigenetic changes such as DNA methylation or histone modification can change the expression of oncogenes too.

Under normal conditions, the mechanisms responsible for genome stability (repair mechanisms) assure that any randomly created mutations are repaired or an abnormal cell is eliminated. In the presence of external mutagens or heritable susceptibility factors such as mutations in tumor suppressor genes, the repair mechanisms malfunction and allow abnormal cells to survive.

The genes involved in the supervision of these processes have been named “**caretaker genes**.” They are responsible for

1. Detecting DNA damage and activating the repair mechanisms

2. Repairing damaged DNA directly

3. Inactivating mutagenic factors

Inactivation of caretaker genes leads to an increased mutation rate and increased genome instability.

The **gatekeeper genes** are responsible for inhibiting tumor growth or promoting death. A mutation in such a gene may promote the development of cancer by decreasing the DNA repair window from G_1 to S phase (see Section III).

B. Tumor-promoting inflammation. It has been recognized that tumors

consist not only of neoplastic cells but also of a variety of cells of the innate and adaptive immune systems. The density of immune cells and their localization within the tumor are different among different malignancies. Initially it was postulated that the presence of these cells reflected an attempt of eradication of a tumor by the immune system. Currently we know that these inflammatory infiltrates can actually promote tumorigenesis and cancer growth by providing cytokines and growth and survival factors for the tumor, by stimulating angiogenesis, and by facilitating invasion and metastasis. Inflammation can ignite the transition from a premalignant state to full malignancy, for example, by release of reactive oxygen species that have mutagenic capabilities.

III. PRINCIPLES OF CANCER CELL GROWTH

A. Normal cell reproduction

1. **The cell cycle** is depicted in Figure 1-1. Cell replication proceeds through a number of phases that are biochemically initiated by external stimuli and modulated by both external and internal growth controls. Certain oncogenes and cell cycle–specific proteins are activated and deactivated synchronously as the cell progresses through the phases of the cell cycle. Most cells must enter the cell cycle to be killed by chemotherapy or radiation therapy. Many cytotoxic agents act at more than one phase of the cell cycle, including those classified as *phase specific.*

   a. In the **G_0 phase** (gap 0 or resting phase), cells are generally programmed to perform specialized functions. An example of drugs that are active in this phase is glucocorticoids for mature lymphocytes.

   b. In the **G_1 phase** (gap 1 or interphase), proteins and RNA are synthesized for specialized cell functions. In late G_1, a burst of RNA synthesis occurs, and many of the enzymes necessary for DNA synthesis are manufactured. An example of drugs that are active in this phase is L-asparaginase.

   c. In the **S phase** (DNA synthesis), the cellular content of DNA doubles. Examples of drugs that are active in this phase are procarbazine and antimetabolites.

d. In the $\mathbf{G_2}$ **phase** (gap 2), DNA synthesis ceases, protein and RNA syntheses continue, and the microtubular precursors of the mitotic spindle are produced. Examples of drugs that are active in this phase are bleomycin and plant alkaloids.

e. In the **M phase** (mitosis), the rates of protein and RNA synthesis diminish abruptly, while the genetic material is segregated into daughter cells. After completion of mitosis, the new cells enter either the G_0 or the G_1 phase. Examples of drugs that are active in this phase are plant alkaloids.

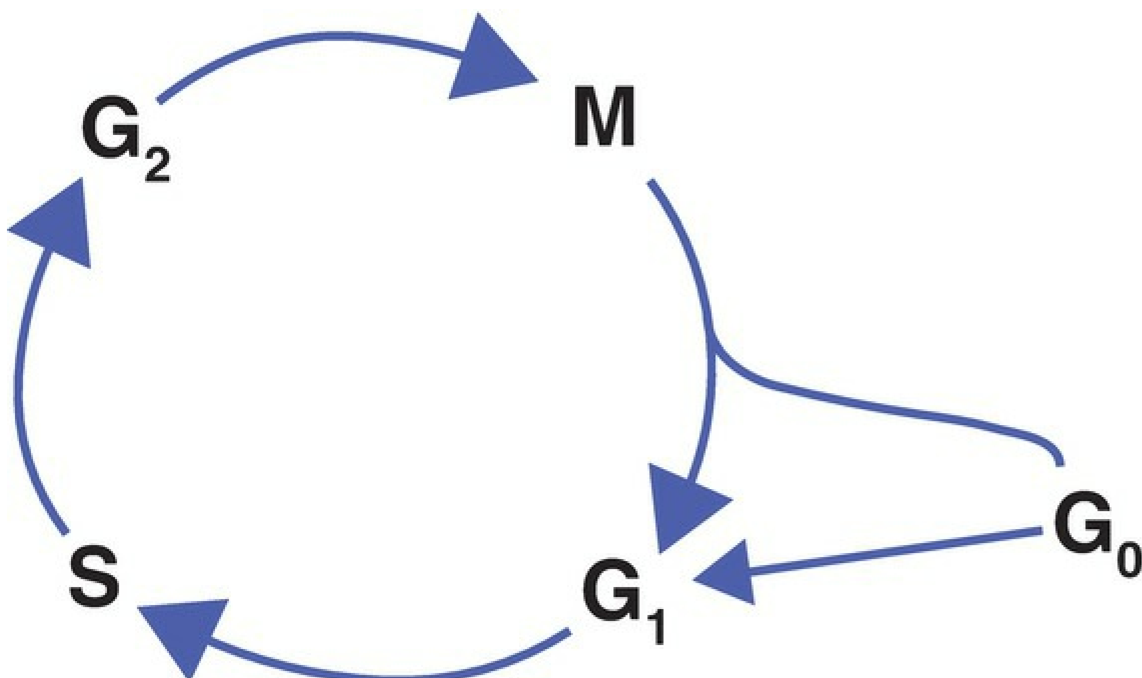

Figure 1-1 Phases of cell growth.

2. Cyclins activate the various phases of the cell cycle. Most normal cells capable of reproduction proliferate in response to external stimuli, such as growth factors, certain hormones, and antigen–histocompatibility complexes, which affect cell surface receptors. These receptors then transduce the signal that results in cell division. Tyrosine kinases (TKs) are an essential part of the cascade of proliferative signals, from extracellular growth factors to the nucleus. Cyclins combine with, activate, and direct the action of special TKs, called *cyclin-dependent kinases*.

3. Cell cycle *checkpoints.* Cells that are capable of reproducing are normally stopped at specific phases of the cell cycle called *checkpoints.* The most important of these are immediately preceding

the initiation of DNA synthesis and immediately preceding the act of mitosis. These histologically quiescent periods are probably mediated by decreased activity of cyclin-associated kinases and tumor suppressor proteins. In fact, the cells in these phases are biochemically active as they prepare proteins to enter the next phase of the cell cycle and correct any genetic defects before going on to reproduce.

a. **Normal cells have mechanisms that detect abnormalities in DNA sequences.** When DNA is damaged, a number of repair mechanisms replace damaged nucleotides with normal molecules. These mechanisms are most important during cellular reproduction to ensure that new genetic material in daughter cells is an exact copy of the parent cell.

b. **The first checkpoint** occurs in the late G_1 phase, just before cells enter the S phase. Even if the proper extracellular signals are received and all of the machinery is in place for DNA synthesis, the DNA must be in an acceptable state, with no lesions, before the cell can leave G_1. If lesions are detected, either they are repaired or the cell is made to undergo apoptosis. This stopping point is one of the actions of the p53 protein.

c. **The second checkpoint** occurs just before the cell enters the M phase; the cell cycle inhibitors stop the cell until it is determined whether the new progenies are worthy successors with accurate genetic copies of the parent. A cell that has not completely and accurately replicated all of its DNA or that does not have the full complement of proteins, spindle materials, and other substances to complete mitosis is arrested at this checkpoint until everything is in order and before the M phase can begin.

4. **Kinetics of tumor growth.** Tumor growth depends on the size of the proliferating pool of cells and the number of cells dying spontaneously. The larger the tumor mass, the greater the percentage of nondividing and dying cells and the longer it takes for the average cell to divide.

a. **The lag phase.** During the earliest phase of tumor development, a small mass of a tumor does not enlarge very much. The working hypothesis about this lag phase is that the “precancer” cells are dividing, but the rate of birth of new cells is offset by cell death. During this phase, the dividing cells are accumulating various

mutations. These mutations help the surviving cells improve their adaptivity to the supply of nutrients, increase the rate at which the mutated cells divide, decrease the rate of apoptosis sensitivity, provide them with invasive properties, make the mutated cells more responsiveness to host factors, and produce angiogenesis factors. Before the angiogenesis factors are expressed, the small tumor does not have its own blood supply and is dependent on local factors to get all the necessary nutrients. Animal models suggested that these tiny cancers may remain unchanged in size and undetectable for many years before they enter the logarithmic phase and are large enough to be detectable.

b. **The log phase.** The tumor now shows rapid exponential growth of the tumor mass. There is a relatively high proportion of cells undergoing division, with rapidly declining rates of cell death, and the *growth fraction* (ratio of dividing to total cells) is high. This rapid growth also reflects the adaptivity of the cells and the production by the tumor cells of angiogenesis factors that induce the surrounding tissues to form new blood vessels that “feed” the tumor mass. When the tumor’s growth fraction is at its highest level, it is still clinically undetectable. Although the reduction in cell number is small, the fractional cell kill from a dose of effective chemotherapy would be significantly higher than later in the course of the tumor.

c. **The plateau phase.** Tumor growth slows down as the percentage of dividing cells decreases, and a larger percentage of cells are dying. The hypotheses are that growth rates eventually plateau because of restrictions of space and nutrient availability, of limitation of blood supply, and of genetic mutations, which cause a higher death rate of cells. The curve becomes asymptotic with some maximum.

IV. THE TUMOR ENVIRONMENT

As discussed before, the tumor consists not only of cancer cells but also of a myriad of cells that are important in cancer growth and survival. The supportive cells interact with cancer cells, but they also interact with each other, assuring the growth advantage for the tumor. This reciprocal interaction remains essential for tumorigenesis. Tumor environment is not a stable structure: it changes as cells progress from potentially cancerous

cells to invasive cancers; different populations of noncancerous cells are found at different stages of cancer. Different cells of the tumor environment are a potential target for anticancer therapies.

A. **Cancer cells** are obviously the most important part of any tumor and they are ultimately responsible for damage caused to the host.
B. **Cancer stem cells (CSCs).** The presence of CSCs is still a controversial notion, and it differs from tumor to tumor. They make a small percentage of all cancer cells, and they are characterized by the ability to reproduce and self-renew and are responsible for generation of a new progeny of cancer cells. They are believed to be more resistant to standard therapies and they are the source of tumor recurrence after achieving initial remission. CSCs are closely related to normal stem cells and share many of the behaviors and features of those normal stem cells. Some theories suggest that CSCs may originate from normal tissue stem cells, other state that they are formed by transdifferentiation of malignant cells. The fact that some tumors may remain dormant for many years after the initial surgery or radiotherapy may be explained by the presence of CSCs. CSCs were first described in hematologic malignancies.
C. **Endothelial cells** are the cells that form tumor-related vasculature. In the presence of VEGF, angiopoietin, and FGF, they are able to enter the tumor and form new blood vessels that are crucial for delivery of nutrients needed for tumor growth. Tumor-associated endothelial cells may have different surface markers from normal endothelial cells and they are a potential target for the therapeutic agents.
D. **Pericytes** are mesenchymal cells that are an essential part of blood vessels; they provide structural and paracrine support. They are a source of Ang-1 that can slow down new blood vessel formation. Possibly tumors with a lower pericyte density have a higher propensity to invade and disseminate.
E. **Immune cells** can play a dual function in the tumor environment: some of them are a part of antitumor response by the immune system, and some actually promote tumor growth by causing chronic inflammation. Various immune cells were implicated in tumorigenesis, including mast cells, macrophages, neutrophils, and even T and B lymphocytes. They can promote angiogenesis, tumor invasion, and metastatic dissemination. In addition, there is a subgroup of bone marrow–derived cells (myeloid derived suppressor cells) and a subgroup of T lymphocytes, named T regulatory cells, that have ability to suppress host immune responses against cancer.

F. Fibroblasts are the main cells that form tumor stroma. A subgroup of fibroblasts—myofibroblasts—enhance cancer proliferation, angiogenesis, invasion, and metastasis.

G. Stromal cells are frequently recruited from surrounding normal tissues. Bone marrow is a source of many mesenchymal cancer stromal cells.

H. Metastatic niche is a term used to describe a permissive environment for cancer cells that disseminate via blood vessels. If such a niche is present, cancer cells do not have to induce the stromal support, but they use preexisting, natural normal tissue stroma. Cancer can promote creation of these niches by secreting cytokines into the blood stream.

V. MOLECULAR AND GENETIC TESTING

Multiple molecular and genetic techniques are currently used in cancer research and in clinical oncology. They are helpful in the discovery process, diagnosis, and prognostic or response assessments and frequently allow physicians to select appropriate patients for appropriate therapy.

A. Detection of mutations. This is normally a targeted testing, and the goal is to detect an expected mutation based on the clinical presentation. Mutations may involve one or multiple genes.

1. Polymerase chain reaction (PCR) is probably the most important molecular tool in research and in clinical molecular testing. It allows for rapid amplification of even trace amounts of DNA. Two primers must be designed in such a way that they can flank the sequence of interest to allow amplification to occur. This technique is not only rapid but also very sensitive (DNA can be amplified from a single cell), specific (even one nucleotide variation can be detected), robust (even DNA of substandard quality can be used), and inexpensive. PCR has a wide clinical utility:

a. Genotyping in particular genomic location allows for detection of point mutations, insertions, deletion, single nucleotide polymorphism (SNP), and some structural variants. It can be used to detect genetic predisposition to cancer, for example, mutations in BRCA1 gene in women with familial breast cancer, or mutations in mismatch repair genes in Lynch syndrome.

b. Detection of rare sequences, for example, detection of herpes virus 8 sequence in samples of Kaposi sarcoma.

c. Quantifying the amount of nucleic acid sequence may be important for monitoring for the presence of residual disease for

example, monitoring of the level of bcr-abl in patients with chronic myelogenous leukemia on therapy with imatinib.

d. **Gene expression profiles** are used for prognostic tests for recurrence of the disease in patients with breast cancer, prostate cancer, and uveal melanoma.

e. **Measurement of viral DNA or RNA** in patients infected with HIV, hepatitis B or C, or other viruses.

2. **Restriction enzyme digestion** is a method that uses bacterial enzymes that are able to cleave a very specific sequence of DNA. If a mutation alters DNA sequence, the digestion would not occur and parts of DNA obtained after digestion would vary in their size. This method cannot be used for disorders characterized by multiple mutations or when mutations occur in the sites not recognized by restriction enzymes. It is used less frequently in clinical oncology.
3. **Amplification refractory mutation system (ARMS)** is a PCR-based method in which two sets of primers are included in one test tube. The test primers are designed in such a way that they bind or do not bind to DNA containing a mutation of interest. The second set of primers is an internal control for the quality of PCR. It can be used in detection of point mutations such as V600E BRAF mutation in melanoma, lung cancer, or colon cancer. It is a fast method and multiple samples can be tested at the same time, but it is not used in cancers with multiple mutations, and unique primers must be designed for each mutation even if it is in the same location, that is, for detection of V600E versus V600K BRAF mutation.
4. **Allele-specific oligonucleotide (ASO) hybridization** is a PCR-based method that uses two membranes on which a PCR product is applied and hybridized with labeled probes that are specific to a mutation on the first membrane and to normal DNA sequence on the second membrane. A panel of mutations for a single patient can be analyzed, but one can detect only one mutation, so it can be used in analysis of tumors with a small number of mutations only.
5. **Genotyping microarrays** can be used to test multiple mutations in one patient or one mutation in multiple patient samples at the same time. This is an automated high-throughput technique with an automated sample analysis; it is expensive and not suitable for testing of a small number of samples in the experimental setting (see Section V).

B. Cytogenetic studies are helpful in cancers that are not characterized by

point mutations or short deletions, but rather by larger structural changes of chromosomes such as translocations, duplications, and isochromosomes (see Appendix A).

1. **Chromosomal analysis.** The cells are arrested in metaphase of their division and individual chromosomes are identified based on their size and banding. It can identify abnormalities in the number of chromosomes and larger structural abnormalities, but it does not detect small losses or gains of DNA.
2. **Fluorescence in situ hybridization (FISH)** can detect large chromosome abnormalities, but probes can be also designed to detect smaller microdeletions or duplications. The probes connected with a fluorescent dye hybridize with a DNA sequence of interest and appear under the microscope as a fluorescent dot. When normal chromosomes are present, two dots should be seen for each pair of chromosomes. If only one is seen, it is equal to a chromosome loss; if more than two are present it shows additional chromosomes. FISH can be used to detect translocations, for example, EWSR-FLI1 in Ewing sarcoma. Probes of two different colors are used: one for each gene of interest. Normally they should be on two different chromosomes. If translocation is present, these dots will overlap. FISH can be used to detect a wide range of structural abnormalities for diagnostic purposes, to serve as a tool for detection of residual disease after therapy.
3. **Spectral karyotyping (SKY)** is a technique based on FISH in which probes of multiple colors are used at the same time to mark all 22 pairs of autosomes and chromosomes X and Y. The readout is computerized. The technique is very expensive, and it cannot detect changes within a single chromosome and small rearrangements.
4. **Comparative genomic hybridization (CGH)** is able to detect amplifications and deletions of smaller DNA regions throughout the whole genome. The tested DNA is compared to the normal one to identify the areas of DNA gains and losses. SNP array is a form of targeted CGH that can help with detection of absence or loss of heterozygosity. The method is not able to detect balanced translocations, inversions, or insertions because they are not associated with a change in copy number.

C. **Genotypic tests** are used when no target mutations are selected before the test is performed. They encompass a larger part of the genome and allow for discovery of unknown or unpredictable variations.

1. **Heteroduplex analysis** and **single-strand conformation analysis (SSCA) are methods** used to detect the presence of point mutations on one strand of DNA, but they cannot detect their location.
2. **Automated sequencing** method uses fluorescently labeled primers or chain terminators to detect the exact sequence of DNA. It can be used to identify precisely not only known or expected mutations but also unknown mutations.
3. **Whole-genome sequencing** does not require identification of a region of interest. The whole DNA sequence is analyzed. Although the method is the most comprehensive, it identifies an increasing number of mutations that are possibly not pathogenic, so-called variants of unknown significance (VUSs). The differentiation between VUSs and mutations responsible for pathogenesis may be challenging.
4. **Southern blotting** is a technique that uses different restriction enzymes to digest DNA into fragments of different length, which are transferred on a membrane and hybridized with a radioactive probe. The location of bands on the membrane corresponds to their size. It can detect a wide range of mutations, but a large amount of DNA is required.

D. **Next-generation sequencing (NGS).** The improvement in technologies led to the development of NGS, in which multiple small fragments of DNA are sequenced in parallel; it expedites the process and decreases its cost. Patient’s purified DNA must be amplified and then fragmented so sequencing can occur. Next, the data are analyzed by computers and aligned against reference DNA, so the whole sequence can be restored from fragments. Third-generation sequencing uses the same concept, but with a single DNA molecule as a template in attempt to reduce the frequency of errors introduced by amplification. NGS can be applied to the whole genome (coding and noncoding DNA), exomes (only DNA encoding proteins is included, 85% of mutations are included in these regions), or even targeted areas of a limited number of genes (10 to 300 genes). Targeted sequencing is faster, is less expensive, and decreases the chance for identification of VUSs.

The clinical applicability of the results of sequencing must be approached with caution: a significant number of nonpathogenic genes are identified and they must be skillfully differentiated from pathogenic genes. The results are frequently compared to the results available in mutation databases, but currently available databases are

not comprehensive enough. An increasing number of medical institutions have molecular tumor boards that attempt to interpret the sequencing results in the context of available knowledge. NGS is the most advanced molecular testing used in the clinic, but ordering of these tests is not always appropriate, especially when the results are unlikely to change the diagnostic or treatment paradigm and when a simpler diagnostic test will lead to the same result.

E. **Gene expression profiling.** The processes of transcription, in which RNA is created on the DNA template, and translation, in which proteins are made based on the code of the messenger RNA (mRNA), are responsible for transferring the information encoded in cancer DNA into proteins, the effector molecules. The molecular analysis of gene expression has been more and more frequently used for disease classification, diagnosis, prognostication, and selection of the treatment. Most of gene expression tests are based on the measurement of mRNA that bridges between DNA and proteins, and they use the microarray technology. Gene expression profile gives information mainly on higher and lower level of gene expression, but it does not give information about mutations or structural changes.

1. **Microarrays (oligonucleotide arrays)** use slides that have short probes synthesized directly on the glass. Patient's mRNA is isolated, reversely transcribed into cDNA, amplified by PCR, transcribed into biotin-labeled cRNA, and hybridized with probes on the glass. Next, fluorophores are applied and scanned by a computerized laser scanner. It allows for obtaining information on a relative level of expression of thousands of genes at the same time; the genes can be identified by their location on the glass.
2. **Transcriptome sequencing** is another method of gene expression profile using the direct sequencing of RNA on the glass.
3. **Clinical applicability.** Currently several gene expression tests are used in the clinic. The 21-gene recurrence score (RS—Oncotype Dx) in patients with breast cancer may identify patients who are most and least likely to derive benefit from adjuvant chemotherapy. Also in breast cancer, other prognostic assays (EndoPredict, Predictor Analysis of Microarray 50 PAM 50, Breast Cancer Index) can be used. Similar RS tests are also available for patients with stage II and III colon cancer and for patients with prostate cancer. The gene expression–based tests predicting the risk of distant metastasis are also available for patients with uveal melanoma and

stage I and II cutaneous melanoma. The number of available tests will continue to grow, but they should be prescribed only if they have been validated and if their result will change the patient management. Assays must be analyzed with an equal scrutiny as the new medications before they are wildly accepted in the clinic.

VI. PRECISION MEDICINE

Precision medicine (also known as personalized medicine) is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person. Launched with a $215 million investment in the President Obama's 2016 Budget, the Precision Medicine Initiative will pioneer a new model of patient-powered research that promises to accelerate biomedical discoveries and provide clinicians with new tools, knowledge, and therapies to select which treatments will work best for which patients. This concept is based on the assumption that better insights into the biologic, environmental, and behavioral influences can lead to better therapeutic options.

A. Why is the precision medicine such a new idea? When one hears about precision medicine, it is possible to believe that, in the past, physicians did not recognize that not every tumor was the same and not every patient was the same. It is obviously not true. We do not treat lung cancer and breast cancer the same. We recognize that estrogen receptor–positive and Her-2/neu-amplified breast cancers are different diseases and we offer different treatments. Physicians and scientists have attempted to be as precise as the current state of knowledge allowed them. The precision medicine initiative brings something new, with the following goals:

1. New technologies were developed, and therefore the testing of cancer is more detailed and more comprehensive.

2. Environmental and genetic factors are included in the selection of the treatment

3. The access to FDA-approved and unapproved medications is supposed to be facilitated. It may require regulatory modernization.

4. The data on the efficacy of medications will be accessible and shared with other investigators. It will assure a success through collaboration.

5. Patients will be more involved in decision making and it will