# Biotechnology and Drug Discovery: From Bench to Bedside

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**Abstract:** New biotechnology and drug discovery technologies are facilitating the rapid expansion of the clinical drug chest, empowering clinicians with a better understanding of disease as well as novel modalities for treating patients. Important research tools and themes include genomics, proteomics, ligand-receptor interaction, signal transduction, rational drug design, biochips, and microarrays. Emerging drug classes include monoclonal antibodies, cancer vaccines, gene therapy, antisense strands, enzymes, and proteins. In this article, we review these topics and illustrate their potential impact by presenting an overview of promising drugs in the pipeline. Clinicians who use these novel treatments must become familiar with these trends.

**Key Words:** biotechnology, drug classes, drug development

Biotechnology is introducing new capabilities to drug discovery, which were considered until recently to be impractical and futuristic. There has been continuous evolution in the integrated approach to the development of therapies in medicine. This effort relies on clinicians, basic scientists, and feedback from novel translational applications.

Initially, biotechnology was synonymous with the emerging recombinant deoxyribonucleic acid (DNA) technology and was used for the large-scale production of proteins, initially "replacement" proteins such as insulin and factor VIII. Later, developments were based on an understanding of ligand-receptor interactions, their impact on disease processes, and the ability to manufacture such large macromolecular proteins for therapeutic purposes. Today, signal transduction and cell signaling and their role in normal and disease states are taking center stage. Small-molecule drug (SMD) discovery, which uses and builds on organic molecules as starting materials, is also benefiting from the input of newer technologies such as combinatorial chemistry and high-throughput screening.

Although many physicians are not exposed to biotechnology, we think that it is valuable for clinicians to gain some fluency in the important trends in this field because the fruits of biotechnological research are reaching the clinic. The speed of events that are occurring in biotechnology is breathtaking and inspiring indeed. The younger generation of physicians has had the privilege of studying molecular biology as medical students. Even those physicians who did study molecular biology in medical school, however, must be excited but somewhat bewildered and uncomfortable about the advent of novel treatment modalities involving the use of antisense strands and monoclonal antibodies (MAb). This review is aimed at practitioners and specialists who are not closely involved in the process of drug discovery and intends to highlight the main developments in biotechnology and their impact on medicine.

## Overview of Biotechnology

Drug discovery and development are costly and complicated processes. More than 99% of experimental compounds

### Key Points

- As a result of new biotechnological capabilities, the understanding of disease processes and the development of new treatments are expanding rapidly.
- Important tools and developments include genomics, proteomics, ligand-receptor interaction, signal transduction, rational drug design, biochips, and microarrays.
- There are several novel drug classes, each with its own structural architecture and mechanism of action, including monoclonal antibodies, cancer vaccines, gene therapy, antisense strands, enzymes, and proteins.
- A wealth of promising new drugs will enable better treatments for patients with cancer, autoimmune disease, neurologic disease, allergy, and transplant rejection, among other entities.

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ultimately fail or are discarded as treatment regimens. Of the chemicals evaluated as part of drug discovery and preclinical testing, only a few proceed to human clinical trials and are approved for marketing.[1] To address this issue, new therapeutic approaches based on genomic and proteomic technology have been developed during the past several years. The *-omic* suffix is an example of the lexicon that has emerged to define the varied populations and subpopulations in the cell. These terms generally carry the *-ome* suffix, with an associated research topic denoted by the *-omics* appellation. The "genome"—that is, the full complement of an organism's genetic information that includes both coding and noncoding DNA sequences—provides a basis for defining the "proteome," which is a list of only the encoding DNA regions that result in protein products.[2] Genomics and proteomics enable the discovery of new genes and proteins and the comparison of their levels in diseased cells, normal cells, and cells treated with compounds that vary in their efficacy and toxicity. Thus, they could prove valuable in identifying new drug targets.

In drug discovery, the drug target is key. A target for pharmaceutical intervention is almost invariably a protein whose function or dysfunction is implicated in a disease process—for instance, growth factors and their receptors, which are frequently overexpressed in carcinomas.[3] A case in point is the epidermal growth factor (EGF) receptor family, which is the most studied growth factor receptor system. These receptors are composed of an extracellular binding domain, a transmembranous lipophilic segment, and an intracellular protein tyrosine kinase domain with a regulatory segment. The interaction of the extracellular growth factor with its receptor (ie, ligand-receptor interaction) results in the activation of cell signaling pathways that lead ultimately to cell division, the synthesis of new proteins, and tumor progression. This cascade of events is known as *signal transduction*. Figure 1

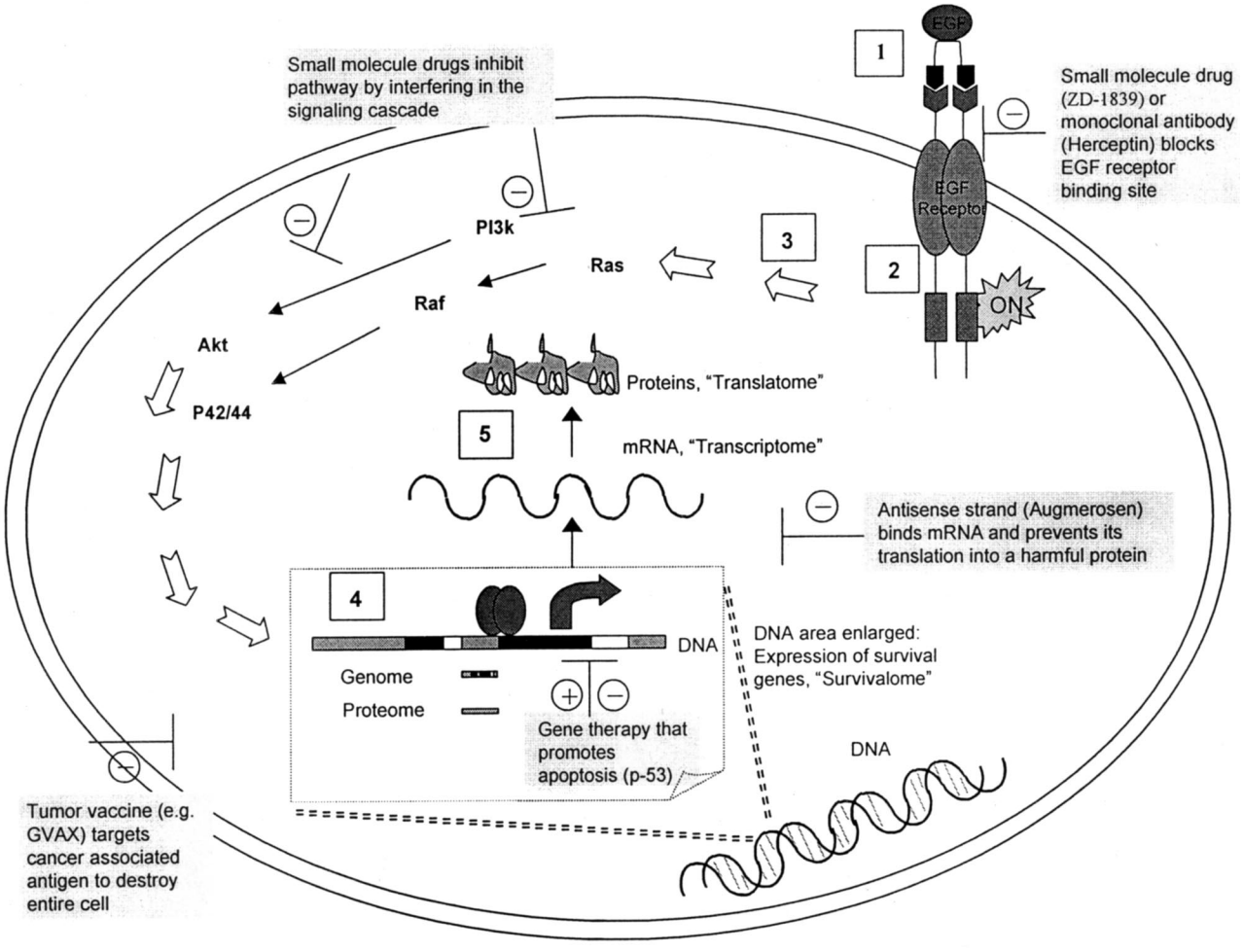


**Fig. 1** **Schematic illustration of the steps along the epidermal growth factor (*EGF*) pathway and potential places for pharmaceutical intervention. Targets for intervention are noted. *Numbers* represent the following steps along the EGF pathway: *1,* binding of EGF to EGF receptor induces dimerization of the receptor; *2,* activation of receptor kinase activity; *3,* interaction of activated receptor with signaling enzymes; *4,* induction of deoxyribonucleic acid (*DNA*) synthesis and transcription into mRNA; *5,* messenger RNA (*mRNA*) is translated to proteins important in inducing further cell growth and survival.**

**Table 1. Small-molecule drug candidates[a]**

| Drug brand name (generic name) | Drug manufacturer/ developer, location | Condition | Clinical trial phase[b] | Comments |
|---|---|---|---|---|
| Tarceva (OSI-774) | OSI Pharmaceuticals, Inc., Melville, NY | Pancreatic, breast, non-small cell lung, and head and neck cancers | III | Selective, orally active inhibitor of ErbB1 tyrosine kinase (an epidermal growth factor receptor), a key oncogene in a variety of cancers, including ovarian, pancreatic, non-small cell lung, breast, and head and neck cancers.[11] |
| Incel (biricodar dicitrate, VX-710) | Vertex Pharmaceuticals, Inc., Cambridge, MA | Ovarian, liver, CAP, and small cell lung cancers; sarcomas | II | Small molecule blocker of multidrug-resistant pumps *MDR1* gene and multidrug resistance-associated protein. Intended to sensitize tumors to chemotherapy.[12] |
| Eflornithine (difluoromethylornithine, or DFMO) | — | Colon and nonmelanoma skin cancers; TCC; Barrett's esophagus | III | Irreversible inhibitor of ornithine decarboxylase, an enzyme elevated in most tumors and premalignant lesions.[13] |
| No brand name (CCI779) | — | Glioma, glioblastoma multiforme, melanoma, small cell lung cancer | II | Binds to the immunophilin FK506-binding protein 12, and the resultant complex inhibits the activity of mammalian target of rapamycin (mTOR).[14] |
| No brand name (atrasentan, ABT-627) | Abbott Laboratories, Abbott Park, IL | CAP | III | Endothelin A receptor inhibitor that inhibits endothelin-1 receptor-mediated effects, including stimulation of prostate cancer proliferation and apoptosis inhibition, and promotes osteoblast activity.[15] (*Note:* Abbott Labs stopped Phase III trial in February 2003.) |
| Velcade (bortezomib, PS-341) | Millennium Pharmaceuticals, Inc., Cambridge, MA | Ovarian, head and neck, and refractory hematologic malignancies, including multiple myeloma and breast cancer | II | Inhibitor of ubiquitin-proteasome pathway that has an important role in cell cycle regulation by degradation of intracellular proteins. This results in shifts in favor of increased cell survival, metastasis, and angiogenesis.[16] Recent report[17] showed that PS-341 has activity against refractory multiple myeloma and possibly non-Hodgkin's lymphoma. |
| Apomine (SR-45023A) | Ilex Oncology, Inc., San Antonio, TX | CAP | II | Bisphosphonate ester that binds to the farnesoid X nuclear receptor. Induces apoptosis of cancer cells.[18] |
| Flavopiridol (HMR1275) | Aventis Pharmaceuticals, Inc., Bridgewater, NJ | Solid tumors and lymphoma | II | A potent inhibitor of cyclin-dependent kinases and others.[19] |
| No brand name (CEP701) | — | CAP | II | Inhibitor of the receptor for nerve growth factor, a tyrosine kinase receptor implicated in cancer pathways.[20] |
| No brand name (GD0039) | GlycoDesign, Inc., Toronto, ON, Canada | Colorectal and breast cancers and RCC | II | Inhibitor of Golgi enzyme mannosidase II, which is responsible for the synthesis of key carbohydrate structures involved in disease.[21] |
| No brand name (UCN-01) | — | Advanced solid tumors | II | Nonspecific inhibitor of many kinases causing cell cycle arrest in G1 and G2 phases in different cell types.[22] |
| Iressa (gefitinib, ZD1839) | AstraZeneca Pharmaceuticals LP, Wilmington, DE | RCC | II | Inhibitor of epidermal growth factor receptor.[23] |
| Thalomid (thalidomide) | Celgene Corp., Warren, NJ | Multiple myeloma, CAP | III | Small molecule antiangiogenesis agent. Also being evaluated in early clinical trials for recurrent or malignant glioma, myelofibrosis with myeloid metaplasia, colorectal cancer, ovarian epithelial cancer, metastatic RCC, and non-small cell lung cancer.[24] |
| No brand name (NV-06, phenoxodiol) | Marshall Edwards, Inc., North Ryde, NSW, Australia | RCC and other solid tumors | II | Signal transduction inhibitor. Blocks enzymes involved in cell division, including topoisomerase-2 and protein tyrosine kinases. Restores apoptosis in cancer cells.[c] |

**Table 1.** *Continued*

| Drug brand name (generic name) | Drug manufacturer/ developer, location | Condition | Clinical trial phase[b] | Comments |
|---|---|---|---|---|
| Reminyl (galantamine HBr) | Janssen Pharmaceutica Products LP, Titusville, NJ | Alzheimer's disease | III | Inhibitor of acetylcholinesterase that also acts agonistically on nicotinic receptors. In clinical trials, patients who took galantamine experienced benefits in cognitive function and activities of daily living.[25] |
| Neovastat (AE-941) | Æterna Laboratories, Inc., Québec City, QB, Canada | Lung and breast cancers and RCC | III | Antiangiogenesis drug (shark cartilage extract) that activates apoptosis of endothelial cells and inhibits the vascular endothelial growth factor-R2 pathway.[26,27] |
| 17-*N*-allylamino-17-demethoxy geldanamycin (17-AAG) | — | Epithelial and hematologic malignancies, and sarcomas | I | Heat shock protein 90 inhibitor. It maintains the conformation, stability and function of key oncogenic proteins involved in signal transduction pathways leading to proliferation, cell cycle progression and apoptosis, as well as other features of the malignant phenotype such as invasion, angiogenesis, and metastasis.[28] |
| No brand name (indiplon, NBI-34060) | Pfizer, Inc., New York, NY, and Neurocrine Biosciences, Inc., San Diego, CA | Insomnia | III | Nonbenzodiazepine agent that acts on a specific site of the γ-aminobutyric acid A receptor in an agonistic fashion.[c] |
| Rotigotine CDS (SPM-962) | Aderis Pharmaceuticals, Hopkinton, MA, and Schwarz Pharma AG, Monheim, Germany | Parkinson's disease | III | Lipid-soluble D2 dopamine agonist in a transdermal delivery formulation (ie, patch). Intended to decrease motor complications associated with chronic use of dopamine agonists, which usually exhibit intermittent levels of dopamine stimulation.[29] |
| No brand name (2-methoxyestradiol) | — | Advanced solid tumors | I | Estrogen-related compound with antiproliferative and antiangiogenic activities.[30] |
| No brand name (ILX295501) | Ilex Oncology, Inc., San Antonio, TX, and Eli Lilly & Co., Indianapolis, IN | Solid tumors | II | Sulfonylurea with antitumor activity against a broad spectrum of solid tumors. Inhibits the enzymes topoisomerase 1 and 2, causing DNA breaks and cell death in cancer cells.[c] |

[a]*CAP, carcinoma of prostate; TCC, transitional cell carcinoma; RCC, renal cell carcinoma; DNA, deoxyribonucleic acid.*

[b]*Phase relates to clinical trial stage in the United States as of April 2003. Some drugs may be in a more advanced clinical trial phase in other countries.*

[c]*Unpublished data.*

illustrates the different steps along the EGF signal transduction pathway that can serve as targets, some of which are being addressed already.

Manifold new drug targets are expected to sprout from the Human Genome Project, which has focused much attention on biotechnology. Indeed, the insight that the Human Genome Project provides with regard to the cell's genetic makeup as well as disease states can be used to understand the cell's protein makeup to generate new protein targets for intervention, such as the EGF family. However, the gene-protein-disease triangle is complex. Therefore, additional contributions from genomic and proteomic technologies is necessary to understand the genetic makeup and expression of diseased cells as well as how the resulting cellular proteins interact to cause disease. For example, biochips are one key technology that enables mutational analysis, gene sequencing, and protein expression testing. They consist of many small arrangements called *microarrays* that contain DNA, ribonucleic acid (RNA), or protein affixed to a small wafer such as that used in computers. Each microarray, or chip, contains thousands of different sequences of nucleotides or proteins. When a gene chip is reacted with a sample of unknown nature, only complementary sequences of DNA bind to the chip; unbound strands are washed away. One illustration of a gene chip's utility is that by using a gene chip with different tumor-associated genes, it is possible to determine whether a mutant gene, or oncogene, is present in a suspected cancer cell. Biochips are thus useful for identifying potential new drug targets.[4,5]

Once a target with a pivotal role in disease is identified, the next step requires designing a drug that will interact with it and deliver a therapeutic effect. Understanding ligand-re-

**Table 2. Protein drug candidates[a]**

| Drug brand name (generic name) | Drug manufacturer/ developer, location | Condition | Clinical trial phase[b] | Comments |
|---|---|---|---|---|
| Plenaxis (Abarelix-Depot, PPI-149) | Praecis Pharmaceuticals, Inc., Waltham, MA | CAP | NDA action date extended 90 days from August 27, 2003, by FDA | Peptide antagonist to gonadotropin-releasing hormone, blocking its action on the pituitary gland.[32] |
| Ampligen (AMP 719) | Hemispherx Biopharma, Inc., Philadelphia, PA | RCC | II/III | Second-generation interferon that acts as an immune system stimulator.[33] |
| No brand name (ABX-EGF) | Abgenix, Inc., Fremont, CA, and Immunex Corp., Seattle, WA | RCC | II | Humanized monoclonal antibody against the epidermal growth factor receptor, which is overexpressed in 70 to 90% of RCCs.[34] |
| Multikine | CEL-SCI Corp., Vienna, VA | CAP | II | Immunotherapeutic agent that is a mixture of interleukins, interferons, and colony-stimulating factors; intratumoral administration.[35] |
| Avicidin | NeoRx Corp., Seattle, WA | Lymphoma, CAP, ovarian, and colon cancers | II | Streptavidin/biotin monoclonal antibody treatment. A multistep delivery system that involves the use of an antibody to target streptavidin to a tumor-associated antigen receptor. Biotin is then used to target the $^{90}Y$ radioisotope to the tumor-localized streptavidin.[36] |
| Natrecor (nesiritide) | Scios, Inc., Sunnyvale, CA | Decompensated CHF | M (FDA approval received 2001) | Recombinant form of B-type natriuretic peptide that produces hemodynamic and symptomatic improvement through balanced vasodilatory effects, neurohormonal suppression, and enhanced natriuresis and diuresis.[37,38] |
| Amevive (alefacept) | Biogen, Inc., Cambridge, MA | Psoriasis | M (FDA approval received January 30, 2003) | Human fusion protein obtained by recombinant DNA technology designed to modulate the activity of T-cells that play a critical role in the pathogenesis of psoriasis and other autoimmune diseases. Alefacept modulates the function of and selectively induces apoptosis of $CD^{2+}$ human memory effector T cells in vivo. Psoriatic plaques are characterized by infiltration with CD45RO+ memory effector T lymphocytes. The recombinant protein alefacept binds to CD2 on memory effector T lymphocytes, inhibiting their activation.[39,40] |
| No brand name (ABX-CBL) | Abgenix, Inc., Fremont, CA, and SangStat Medical Corp., Fremont, CA | GVHD | II/III | A murine immunoglobulin M monoclonal antibody that recognizes CD147 on the cell surface. Human CD147, also known as neurothelin or extracellular matrix metalloproteinase inducer, is a member of the immunoglobulin superfamily. On activation, CD147 is upregulated on T and B lymphocytes, which are then depleted through a complement-dependent cytotoxic mechanism.[41] |
| Xolair (omalizumab) | Genentech, Inc., South San Francisco, CA, Novartis AG, East Hanover, NJ | Asthma and seasonal allergies | M (FDA approval received June 2003) | A novel humanized monoclonal antibody directed against the high-affinity FcεRI portion of the immunoglobulin E. It decreases serum immunoglobulin E levels in a dose-dependent manner. It was shown to significantly reduce the rate of exacerbation and improve disease control in patients at high risk for serious asthma-related morbidity and mortality.[42] |
| No brand name (epratuzumab, AMG 412) | Amgen, Inc., Thousand Oaks, CA | Non-Hodgkin's lymphoma | III | Humanized anti-CD22 monoclonal antibody. CD22 is commonly expressed by B-cell malignant cells.[43] |

**Table 2.** *Continued*

| Drug brand name (generic name) | Drug manufacturer/ developer, location | Condition | Clinical trial phase[b] | Comments |
|---|---|---|---|---|
| Bexxar (tositumomab and $^{131}$I-tositumomab) | Corixa Corp., Seattle, WA, and GlaxoSmithKline, Research Triangle Park, NC | Non-Hodgkin's lymphoma | M (FDA approval received June 30, 2003) | Anti-CD20 murine monoclonal antibody conjugated with radioactive iodine ($^{131}$I); radioimmunotherapy.[44] |
| Antegren (natalizumab) | Biogen, Inc., Cambridge, MA, and Elan Corp., plc, Dublin, Ireland | MS, Crohn's | III | Humanized monoclonal antibody and the first in a new class of potential therapeutics known as $\alpha$4 integrin inhibitors that are designed to block immune cell adhesion to blood vessel walls and subsequent migration of lymphocytes into tissue.[45,46] |
| Keyhole Limpet Hemocyanin (KLH, DRG-0070) | — | Superficial bladder TCC | III | Immune modulator protein derived from the mollusk *Megathura crenulata* (great keyhole limpet), which is found along the coast of California and Mexico.[47] |

[a]*CAP, carcinoma of prostate; NDA, new drug application to U.S. Food and Drug Administration (FDA); RCC, renal cell carcinoma; CHF, congestive heart failure; DNA, deoxyribonucleic acid; GVHD, graft versus host disease; MS, multiple sclerosis; TCC, transitional cell carcinoma; M, approved medication.*

[b]*Phase relates to clinical trial stage in the United States as of April 2003. Some drugs may be in a more advanced clinical trial phase in other countries.*

ceptor interaction is a key element in designing a drug to interact with a target. To bind with the target, most drug molecules insert themselves into a functionally critical site of the target protein, like a key in a lock. The molecule then either induces or, more commonly, inhibits the protein's function. Thus, a better understanding of the target's structure and functionality is key to designing better therapeutics, or ligands, that bind to the target. In recent years, better understanding of protein structure and function has yielded sophisticated approaches to the generation and optimization of drug candidates. These methods are commonly referred to as *rational drug design*. In essence, rational drug design tailors drug candidates to their target proteins by first elucidating the three-dimensional structure, the binding site, and the active site of the target.[6] Next, medicinal chemists apply combinatorial chemistry techniques and high-throughput screening tools to generate large libraries of compounds whose structure corresponds to the target's strategic site. With the use of biologic assays that reflect the activity of the target protein, researchers can modify the drug candidate to achieve the ideal in vitro effect and test antitargets to determine the drug's specificity.

## Biotechnology Drug Classes and Selected Drugs in the Pipeline

A snapshot of some drug candidates in development is beneficial to the understanding of how the technologies discussed in this article are used in drug development and in highlighting areas in medicine in which they may have a significant impact in the near future. We analyzed publicly available information, including the medical literature as well as U.S. Food and Drug Administration (FDA) and drug company reports, to generate a representative but by no means exhaustive list of drug candidates currently in clinical trials. To facilitate this discussion, we found it valuable to assign drugs into classes according to their chemical composition.

***Small-Molecule Drugs.*** SMDs normally have limited biologic interaction capability and less specificity than other drugs for desired targets. In general, an SMD acts as a "spoiler," because its therapeutic effect is limited to the inhibition of an effector protein. For example, by interacting with a hormone receptor, the SMD can inhibit the binding activity of the respective hormone by occupying its docking site or by causing a change in the receptor's three-dimensional configuration. Both patients and the pharmaceutical industry favor the use of SMDs rather than other modalities because of their attractive pharmacokinetic properties, especially their suitability for oral administration and ease of development.[7] SMDs are well positioned to target intracellular proteins (ie, enzymes), because cell membrane penetration is often feasible.

The tyrosine kinase inhibitor STI571 (imatinib mesylate, Gleevec; Novartis Pharmaceuticals Corp., East Hanover, NJ) is an SMD that has had an exceptional impact on the management of Philadelphia-positive chronic myelogenous leukemia (ie, Bcr-Abl-positive) and gastrointestinal stromal tumors with Kit mutations.[8,9] STI571 was first developed to target the platelet-derived growth factor receptor but then was found to be an inhibitor of a specific target protein: the Bcr-Abl protein kinase. Bcr-Abl had previously been implicated in the pathogenesis of Philadelphia mutation-positive leukemia, and STI571 was then developed through a rational process of screening and refining potential small molecules. It therefore serves as a fitting example of the way in which rational drug design is effecting drug development and dis-

ease management.[10] Selected SMDs that are currently in clinical trials for different applications are listed in Table 1.[11–30]

***Protein Drugs.*** Protein drugs can be subdivided according to their mechanism of action. This categorization scheme helps to create order in the world of protein drugs, yet overlaps exist between different subtypes. Until the early 1970s, proteins were derived from animals or were manufactured synthetically. This process greatly limited their use as pharmaceuticals because of availability and cost issues. Recombinant DNA technology significantly changed these circumstances. Scientists could now insert into a bacterial or yeast cell the human DNA comprising a gene and use the host to manufacture the human protein. With the use of that technology, the bottleneck in protein therapeutics shifted from protein manufacturing to the identification of drug targets and the generation of drug candidates.

***Therapeutic Hormones and Enzymes.*** Hormones have been an important subject in pharmaceutical research, because the biology of various hormone deficiencies is relatively straightforward, and thus animal models can be created for research purposes. For instance, insulin was discovered in 1921, leading to the advent of replacement therapy with porcine insulin and a better understanding of diabetes. Furthermore, because hormones are circulating entities, they are more accessible than other proteins—especially intracellular proteins—for research purposes. Hence, early protein therapeutics targeted hormone deficiency states, and when recombinant DNA technology emerged, it was used to manufacture replacement proteins, whose role in disease was relatively clear. Enzymes have enormous potential to serve as pharmaceuticals because of their vast number and ubiquitousness. That many enzymes operate intracellularly poses practical problems, however, because protein delivery into the cell is currently next to impossible. More intracellular enzymes are continually being discovered, and methods of enabling oral and intracellular protein delivery are among the most burning challenges in biotechnology.[31] Notable protein drugs that are in the advanced development to launch stages include a T cell-modulating fusion protein for patients with psoriasis and various other autoimmune diseases, as well as recombinant natriuretic peptide for the treatment of patients with congestive heart failure (see Table 2).[33–47]

***Monoclonal Antibodies.*** Antibodies (immunoglobulins) are proteins manufactured by B-lymphocytes. They consist of a highly diverse binding site known as the variable region (Fab), which sticks to a corresponding antigen, and a crystallizable fragment domain (Fc), which determines the antibody's functionality. MAb are products of a distinct clone of B cells and are usually derived by immunizing mice against the desired antigen. The reactive B cells are fused with myeloma cells to create hybridomas, which are essentially clones of immortalized B cells that share specificity for the same antigen.[48] The hybridomas' identical product antibody is called a *monoclonal antibody,* or MAb.

The ability to manufacture clones of identical MAb enables different strategies to create MAb therapeutics. By recruiting the immune system's lytic action, MAb lead to the destruction of antigens implicated in disease. MAb can be used to target toxic drugs such as chemotherapy directly at their site of action, thereby reducing side effects and the required dosage. MAb can be used as diagnostic agents to locate, for instance, residual tumor cells after surgery.[49]

In recent years, MAb have made the leap from promising investigational therapies to the clinic, and currently there are 12 approved MAb in the United States. Eight of those 12 MAb are approved for therapeutic indications, including cancer, autoimmune disease, viral infection, and myocardial infarction. In addition, four MAb have been approved for use as diagnostic agents. Most of these have had a significant impact on disease, as evidenced in the discussion of selected MAb in the next few paragraphs.

Rituximab (Rituxan; Genentech, Inc., South San Francisco, CA), the first MAb approved for the treatment of cancer patients, is indicated for non-Hodgkin's B cell lymphoma. It is directed against the cytosine deaminase 20 (CD20) protein, which is found on the surface of normal and malignant B cells. As a single agent, rituximab induces meaningful responses in approximately one-half of patients with relapsed indolent lymphomas and in approximately one-third of patients with relapsed aggressive lymphomas. Because it is a nonchemotherapeutic agent, it also presents a relatively benign side effect profile. Rituximab is currently being tested for other B cell disorders.[50,51]

Infliximab (Remicade; Centocor, Inc., Malvern, PA) is a chimeric (human-mouse) MAb that neutralizes tumor necrosis $\alpha$, a proinflammatory cytokine. It is approved for the treatment of patients with rheumatoid arthritis and Crohn's disease. In clinical trials for rheumatic arthritis, infliximab produced significant improvements in all measures of disease, and treatment with infliximab combined with methotrexate was found to be superior to treatment with methotrexate alone.[52] In Crohn's disease, clinical trials showed infliximab to be effective in producing and maintaining a clinical response in patients with refractory moderate to severe disease.[53]

Trastuzumab (Herceptin; Genentech, Inc.) is a humanized MAb that targets the extracellular portion of the human epidermal growth factor receptor 2 (HER2)/Neu receptor. The latter is a member of the EGF receptor family, the blockade of which inhibits the growth of tumors that express it. HER2/Neu is overexpressed in 25 to 30% of breast cancers, increasing tumor aggressiveness. In HER2/Neu-positive patients, the use of trastuzumab with chemotherapy was associated with improved time until disease progression and with overall survival.[54] Several promising MAb currently in late-stage clinical trials are targeting non-Hodgkin's lymphoma, asthma, psoriasis, and different solid organ tumors (Table 2).[33–47]

***Cytokines.*** Cytokines are proteins that regulate cells that belong to the immune system, such as lymphocytes and mac-

rophages. Cytokines have a pivotal role in normal and disease mechanisms in which immune processes play a role, including chronic infectious, autoimmune, cancer, and coronary heart diseases. Many cytokines and cytokine inhibitors are available or are being developed as therapeutics.[55–59]

Interleukins (ILs) and interferons are a large and varied family of compounds produced by lymphocytes, macrophages, and monocytes. The FDA has approved the use of recombinant IL-2 for the treatment of patients with renal cell carcinoma, but IL-2 is highly toxic because of its central role in the immune system, which thus far has limited its impact.[60] The FDA has approved the use of recombinant interferons for patients with human immunodeficiency virus-related Kaposi's sarcoma, genital warts, hairy cell leukemia, and hepatitis B and C.[59]

Colony-stimulating factors (CSFs) stimulate bone marrow stem cells to differentiate toward a particular cell type. Recombinant versions of CSF, including granulocyte-macrophage CSF, granulocyte CSF, and erythropoietin have revolutionized the ability to treat myelosuppression. Most notably, these agents have had a significant impact in cancer treatment, in which myelosuppression is a common complication of chemotherapy. Proved and suggested effects of treatment include shortening the duration of febrile neutropenia after myelosuppressive treatment, mobilization of hematopoietic stem cells for ensuing transplantation, and reductions in chemotherapy-associated infections, antibiotic use, hospital stay, and mortality.[61,62] Erythropoietin has had a profound effect on the treatment of patients with end stage renal failure-associated anemia.[63] Some investigational cytokines are listed in Table 2.

***Gene Therapy.*** Gene therapy may be defined as the transfer of recombinant DNA into human cells to achieve the production of a desired protein. Depending on the strategy used, DNA may be introduced into cells removed from the body (ie, the ex vivo approach) or directly into cells in their normal location (ie, the in vivo approach).[64] Gene therapy has various potential applications, such as treating patients with enzyme deficiencies or cancer. Efficient gene transfer requires the use of a vector. All vectors contain, at a minimum, the transgene of interest linked to a promoter to drive its expression.[65] Increasingly wider ranges of viral and synthetic vectors are available, each of which has characteristic advantages and limitations. Generally, viral vectors achieve better transfection than other vectors but have other problems such as immunogenicity and complicated manufacturing. Liposomes are nonviral vectors that mitigate the immunogenicity problem but provide less efficient transfection and protein expression. Naked DNA is a third method that uses plasmids, which usually are administered by direct injection into tumor or muscle as opposed to systemic delivery.[66]

There are several strategies whereby gene therapy may be used to treat cancer. In the corrective gene therapy approach, when malignant transformation is associated with inactivity of tumor suppressor genes such as *p53* and *p21,* supplying tumors with the intact gene may reverse malignant transformation by promoting apoptosis.[67–69] Another strategy is cytoreductive gene therapy, in which immunotherapy or cytolytic/proapoptotic approaches are used. Immunotherapy uses gene transfer to facilitate a dormant host immune response directed against the tumor. Evasion of autologous host cellular immunity is a common feature of tumor cell neoantigens, because tumor cells are poor antigen-presenting cells. Cancer vaccine strategies are based on optimization of the context in which tumor antigens or tissue-specific antigens are presented to the host immune system. When appropriately primed, the activated host immune system can then act against tumor cells systemically.

The ex vivo approach starts with inactivated cancer cells obtained from the patient. Different techniques are then used to enhance the immunogenicity of tumor-specific antigens, including growth in a cytokine-rich environment, coinjection of tumor cells along with cytokines back into the patient, or transfection of these cells in vitro with genes that encode immunostimulatory cytokines. A second approach is to administer an injection of a purified tumor-associated protein or peptide into the patient, without injecting the entire tumor cell. The third approach uses in vitro manipulation of host antigen-presenting cells such as dendritic cells, which are involved in initiating the T cell-mediated response against antigens. Confronting dendritic cells with the desired antigen in vitro stimulates an immune response upon injection into the patient.[70,71] Enzyme/prodrug gene therapy, also referred to as *suicide gene therapy,* relies on the conversion of an inactive prodrug into a toxic drug with the use of an enzyme vectored only to the target tumor cells. In this way, active drug is limited spatially to the transduced cells and adjacent surrounding cells, facilitating higher tumor drug concentrations without increased normal tissue toxicity. Prodrug-activating enzymes that have been used in this approach include cytosine deaminase, which catalyzes the conversion of the nontoxic 5-fluorocytosine to the cytotoxic 5-fluorouracil, and herpes simplex virus thymidine kinase, which, together with cellular enzymes, facilitates the conversion of ganciclovir into the toxic ganciclovir triphosphate.

Viral vectors may themselves be designed to target and kill tumor cells without the insertion of a foreign transgene (eg, oncolytic viruses). The adenovirus life cycle includes a lytic phase, which can result in host cell death independent of entry into the cell cycle. Adenovirus has evolved a potent repertoire of gene products that may exert profound effects on the growth regulation of the host cell to facilitate viral replication. The ONYX-015 vector, a replication-competent adenovirus designed to preferentially replicate in p53 mutant cells, is currently in clinical trials (Table 3).[72] ONYX-015 is the first genetically engineered replication-competent virus to demonstrate selective intratumoral replication and necrosis in patients.[73]

***Antisense Drugs.*** Although traditional drugs are designed to interact with protein molecules, antisense drugs are de-

**Table 3. Cancer vaccines and gene therapy drug candidates[a]**

| Drug brand name (generic name) | Drug manufacturer/ developer, location | Disease | Clinical trial phase[b] | Type | Comments |
|---|---|---|---|---|---|
| Advexin (INGN 201, Ad5CMVp53) | Introgen Therapeutics, Inc., Austin, TX | Head and neck, breast, lung, and pancreatic cancers and TCC | II/III (FDA designated Fast Track Drug Product status September 2003) | Gene therapy | Gene therapy applying the *p53* gene using an adenoviral vector. Most advanced clinical trials are for head and neck cancers, whereas the same approach is in earlier trials for other oncologic indications.[74,75] |
| OncoVAX | Intracel, LLC, Frederick, MD | Colorectal cancer and RCC | II | Cellular cancer vaccine | Irradiated autologous cancer cells obtained in surgery are attached to Bacille bilié de Calmette-Guérin vaccine and returned to patient to stimulate immune response.[76] |
| Leuvectin | Vical, Inc., San Diego, CA | RCC and CAP | II | Gene therapy | Gene therapy based on the IL-2 gene. Evaluated for metastatic RCC and local CAP. Administered as direct intratumoral injection.[77] |
| Oncophage (HSPPC-96) | Antigenics, Inc., New York, NY | Lung cancer and RCC melanoma | III | Peptide cancer vaccine | Vaccine consisting of autologous heat shock protein-peptide complexes derived from patient's own tumor tissue.[78] |
| Genasense (oblimersen sodium, G-3139; formerly augmerosen) | Genta, Inc., Berkeley Heights, NJ | CLL, AML, melanoma, MM, and CAP | II/III | Antisense | An antisense oligonucleotide specific for Bcl-2 that is expressed by most types of cancer.[79] |
| Provenge | Dendreon Corp., Seattle, WA | CAP | III | Cellular cancer vaccine | Therapeutic cellular vaccine for hormone-resistant CAP. Based on sensitizing patient's dendritic cells to a ubiquitous CAP antigen.[80,81] |
| GVAX | Cell Genesys, Inc., South San Francisco, CA | CAP, lung, and pancreatic cancers and AML | II/III | Cellular cancer vaccine | Non-patient-specific cancer vaccine composed of irradiated tumor cells modified to secrete GM-CSF. Administered intradermally.[80,82] |
| Avicine | AVI BioPharma, Inc., Portland, OR | CAP | II | Peptide cancer vaccine | Cancer vaccine designed to induce an immune response against the hormone human chorionic gonadotropin, which is present in most prostate carcinomas. Consists of a short peptide part of human chorionic gonadotropin attached to the diphtheria toxoid.[83] |
| Prostvac | Therion Biologics Corp., Cambridge, MA | CAP | II | Gene therapy | DNA vaccine based on *PSA* gene with a gene for an adjuvant attached, using a vaccinia virus vector.[84] |
| CG7060 (formerly CV706) | Cell Genesys, Inc., South San Francisco, CA | CAP | I/II | Gene therapy | Adenovirus engineered to grow in cancer cells expressing *PSA*.[85] |
| CG7870 (formerly CV787) | Cell Genesys, Inc., South San Francisco, CA | CAP | I/II | Gene therapy | Adenovirus engineered to grow in cancer cells expressing *PSA*.[86] |
| TG4010 (MVA-MUC-IL2) | Transgene, S.A., Strasbourg, France | CAP | I | Gene therapy | Gene therapy using the genes for tumor-specific antigen MUC-1 and for IL-2, delivered by a vaccinia (ie, pox) virus vector.[87] |
| INGN 201 AD-p53 | Introgen Therapeutics, Inc., Austin, TX | CAP, TCC | II | Gene therapy | *P53* gene therapy using an adenoviral vector. A similar product is in Phase III clinical trials for head and neck cancer.[88] |
| Prostate-specific membrane antigen (PSMA) vaccine | PSMA Development Co., LLC (Progenics Pharmaceuticals, Inc., Tarrytown, NY, and Cytogen Corp., Princeton, NJ, in collaboration with AlphaVax Human Vaccines, Inc., Research Triangle Park, NC) | CAP | II | Cellular cancer vaccine | Cancer vaccine based on the PSMA epitope found in CAP cells using ex vivo dendritic cell processing.[89] |

**Table 3.** *Continued*

| Drug brand name (generic name) | Drug manufacturer/ developer, location | Disease | Clinical trial phase[b] | Type | Comments |
|---|---|---|---|---|---|
| Anti-CEA vaccine | Dendreon Corp., Seattle, WA | RCC, TCC, CAP, and other | I | Cellular cancer vaccine | Patient T cells modified in vitro with anti-CEA immunoglobulin to serve as T cell receptors and reinfused in patients with CEA plus tumors.[c] |
| Vitravene (fomivirsen) | Isis Pharmaceuticals, Inc., Carlsbad, CA | CMV retinitis in patients with AIDS | M | Antisense | Antisense CMV inhibitor complementary to mRNA of major immediate-early region proteins of CMV. It was found to be a potent and selective antiviral agent for CMV retinitis.[90,91] |
| Angiozyme | Ribozyme Pharmaceuticals, Inc., Boulder, CO, and Chiron Corp., Emeryville, CA | Colon and breast cancers | II | Antisense | Inhibits the VEGF receptor, a key component in angiogenesis. This ribozyme is a catalytic RNA molecule that downregulates VEGF receptor function by specifically cleaving the mRNAs for the primary VEGF receptors Flt-1 and KDR.[92] |
| Onyx-015 | Onyx Pharmaceuticals, Inc., Richmond, CA, and XOMA (US) LLC, Berkeley, CA | Head and neck, pancreatic, colorectal, and lung cancers | III | Gene/viral therapy | Recombinant adenovirus engineered to replicate only in cells expressing a *p53* mutation. The virus carries a loss-of-function mutation that disables inactivation of tumor suppressor protein p53. Thus, in normal cells, p53 accumulates and inhibits viral replication, whereas cancer cells with a *p53* mutation allow viral replication and cell death.[72,93] (*Note:* Drug development discontinued in January 2003 pending developers' ongoing partnership discussions.) |
| Generx (Ad5FGF4) | Schering AG, Berlin, Germany | Stable coronary artery disease | II/III | Gene therapy | Adenovirus vector containing human fibroblast growth factor-4 planned to trigger the production of new blood vessel growth.[94] |

[a]*TCC, transitional cell carcinoma; RCC, renal cell carcinoma; CAP, carcinoma of prostate; CEA, carcinoembryonic antigen; CLL, chronic lymphatic factor; AML, acute myelogenous leukemia; MM, multiple myeloma; GM-CSF, granulocyte monocyte colony stimulating factor; mRNA, messenger ribonucleic acid; M, approved drug; CMV, cytomegalovirus; VEGF, vascular endothelial growth factor.*

[b]*Phase relates to clinical trial stage in the United States as of April 2003. Some drugs may be in a more advanced clinical trial phase in other countries.*

[c]*Unpublished data.*

signed to inhibit the production of disease-causing proteins. During the transcription of information from DNA to messenger RNA (mRNA), two complementary strands of DNA partly uncoil such that one strand is used as a template for the transcribing enzymes, which assemble mRNA in a process called *transcription*. mRNA then migrates into the cell, where its encoded information is read by the ribosomes and translated to the specific protein.[95]

Antisense drugs are complementary strands of small segments of mRNA. To create antisense drugs, nucleotides are linked in short chains (ie, oligonucleotides). Each antisense drug is designed to bind to a specific sequence of nucleotides in its mRNA target to inhibit the production of the protein encoded by the target mRNA. Fomivirsen (Vitravene; Isis Pharmaceuticals, Inc., Carlsbad, CA) is an antisense strand complementary to the mRNA of a crucial cytomegalovirus protein. It is an FDA-approved medication indicated for patients with acquired immunodeficiency syndrome-related cytomegalovirus retinitis. Oblimersen sodium (Genasense; Genta, Inc., Berkeley Heights, NJ) is an antisense drug that binds to the Bcl-2 mRNA, which is expressed by different cancers (Table 3).

## Conclusions

The development of a new drug requires the identification of a protein target, techniques for the generation of compounds that react with the target in a desired fashion, and innovative delivery mechanisms by which to lead the drug to its target. The tools of biotechnology are effecting advancements on all of these fronts. An abundance of new gene and protein targets that can be targeted by therapeutics are being investigated. Moreover, whereas in the past most drugs were randomly generated small-molecule compounds that were

limited to the blockade of certain pathways, other drug classes have emerged, including recombinant protein drugs and MAb, DNA and cellular vaccines, gene therapy, and antisense therapy. SMDs, which remain a fundamental weapon against many diseases, can be engineered to provide a better therapeutic profile than before. Some of the newer drug classes, including protein drugs and MAb, already have exhibited proof of concept as approved drugs on the basis of several years of experience. The potential impact on disease of cancer vaccines, gene therapy, and antisense therapy remains to be determined, but there seems to be consensus regarding the eventual important role of these technologies.

As a result of these developments, physicians will be able to attack the same target with a mix of various drug classes, such as combinations of MAb or a cancer vaccine against a tumor-associated protein, a cytokine to increase the antitumor immune response, and gene therapy encoding for a suicide protein. Such an approach is not yet feasible, but the drugs that will allow experimentation with such combinations are at our doorstep.

Several drug candidates are far along the route to becoming FDA-approved drugs. These include a cellular vaccine for hormone-resistant prostate cancer, a cancer vaccine and a small-molecule antiangiogenic drug for renal cell carcinoma, a new immune modulator protein drug directed against psoriasis and other autoimmune diseases, an SMD directed against transitional cell carcinoma, and several MAb that target cancer, autoimmune disease, and graft versus host disease. Judging from the wealth of advanced clinical candidates in the pipeline, the impact of biotechnology on the practice of medicine will soon increase markedly, empowering clinicians with new ways to fight disease.

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## References

1. Cunningham MJ. Genomics and proteomics: The new millennium of drug discovery and development. *J Pharmacol Toxicol Methods* 2000; 44:291–300.
2. Greenbaum D, Luscombe NM, Jansen R, Qian J, Gerstein M. Interrelating different types of genomic data, from proteome to secretome: 'oming in on function. *Genome Res* 2001;11:1463–1468.
3. Sebti SM, Hamilton AD. Design of growth factor antagonists with antiangiogenic and antitumor properties. *Oncogene* 2000;19:6566–6573.
4. Jain KK. Biochips for gene spotting. *Science* 2001;294:621–623.
5. MacBeath G, Schreiber SL. Printing proteins as microarrays for high-throughput function determination. *Science* 2000;289:1760–1763.
6. Blundell TL. Structure-based drug design. *Nature* 1996;384:23–26.
7. Garrett MD, Workman P. Discovering novel chemotherapeutic drugs for the third millennium. *Eur J Cancer* 1999;35:2010–2030.
8. Savage DG, Antman KH. Imatinib mesylate: A new oral targeted therapy. *N Engl J Med* 2002;346:683–693.
9. Tuveson DA, Willis NA, Jacks T, Griffin JD, Singer S, Fletcher CD, et al. STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: Biological and clinical implications. *Oncogene* 2001;20: 5054–5058.
10. Capdeville R, Buchdunger E, Zimmermann J, Matter A. Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nat Rev Drug Discov* 2002;1:493–502.
11. de Bono JS, Rowinsky EK. The ErbB receptor family: A therapeutic target for cancer. *Trends Mol Med* 2002;8:S19–26.
12. Rowinsky EK, Smith L, Wang YM, Chaturvedi P, Villalona M, Campbell E, et al. Phase I and pharmacokinetic study of paclitaxel in combination with biricodar, a novel agent that reverses multidrug resistance conferred by overexpression of both MDR1 and MRP. *J Clin Oncol* 1998;16:2964–2976.
13. Crowell JA, Goldenthal EI, Kelloff GJ, Malone WF, Boone CW. Chronic toxicity studies of the potential cancer preventive 2-(difluoromethyl)-DL-ornithine. *Fundam Appl Toxicol* 1994;22:341–354.
14. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: Involvement of vascular endothelial growth factor. *Nat Med* 2002;8:128–135.
15. Samara E, Dutta S, Cao G, Granneman GR, Dordal MS, Padley RJ. Single-dose pharmacokinetics of atrasentan, an endothelin-A receptor antagonist. *J Clin Pharmacol* 2001;41:397–403.
16. Adams J. Proteasome inhibition in cancer: development of PS-341. *Semin Oncol* 2001;28:613–619.
17. Orlowski RZ, Stinchcombe TE, Mitchell BS, Shea TC, Baldwin AS, Stahl S, et al. Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. *J Clin Oncol* 2002;20:4420–4427.
18. Alberts DS, Hallum AV III, Stratton-Custis M, Garcia DJ, Gleason-Guzman M, Salmon SE, et al. Phase I pharmacokinetic trial and correlative in vitro phase II tumor kinetic study of Apomine (SR-45023A), a novel oral biphosphonate anticancer drug. *Clin Cancer Res* 2001;7:1246–1250.
19. Zhai S, Senderowicz AM, Sausville EA, Figg WD. Flavopiridol, a novel cyclin-dependent kinase inhibitor, in clinical development. *Ann Pharmacother* 2002;36:905–911.
20. George DJ, Dionne CA, Jani J, Angeles T, Murakata C, Lamb J, et al. Sustained in vivo regression of Dunning H rat prostate cancers treated with combinations of androgen ablation and Trk tyrosine kinase inhibitors, CEP-751 (KT-6587) or CEP-701 (KT-5555). *Cancer Res* 1999; 59:2395–2401.
21. Goss PE, Baker MA, Carver JP, Dennis JW. Inhibitors of carbohydrate processing: A new class of anticancer agents. *Clin Cancer Res* 1995;1: 935–944.
22. Monga M, Sausville EA. Developmental therapeutics program at the NCI: Molecular target and drug discovery process. *Leukemia* 2002;16: 520–526.
23. Sirotnak FM, Zakowski MF, Miller VA, Scher HI, Kris MG. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. *Clin Cancer Res* 2000;6:4885–4892.
24. D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci U S A* 1994;91:4082–4085.
25. Wilkinson D, Murray J. Galantamine: A randomized, double-blind, dose comparison in patients with Alzheimer's disease. *Int J Geriatr Psychiatry* 2001;16:852–857.
26. Gingras D, Renaud A, Mousseau N, Beliveau R. Shark cartilage extracts as antiangiogenic agents: Smart drinks or bitter pills? *Cancer Metastasis Rev* 2000;19:83–86.
27. Gingras D, Renaud A, Mousseau N, Beaulieu E, Kachra Z, Beliveau R. Matrix proteinase inhibition by AE-941, a multifunctional antiangiogenic compound. *Anticancer Res* 2001;21:145–155.

28. Workman P, Maloney A. HSP90 as a new therapeutic target for cancer therapy: The story unfolds. *Expert Opin Biol Ther* 2002;2:3–24.
29. Metman LV, Gillespie M, Farmer C, Bibbiani F, Konitsiotis S, Morris M, et al. Continuous transdermal dopaminergic stimulation in advanced Parkinson's disease. *Clin Neuropharmacol* 2001;24:163–169.
30. Hughes RA, Harris T, Altmann E, McAllister D, Vlahos R, Robertson A, et al. 2-Methoxyestradiol and analogs as novel antiproliferative agents: Analysis of three-dimensional quantitative structure-activity relationships for DNA synthesis inhibition and estrogen receptor binding. *Mol Pharmacol* 2002;61:1053–1069.
31. Koths K. Recombinant proteins for medical use: The attractions and challenges. *Curr Opin Biotechnol* 1995;6:681–687.
32. Cook T, Sheridan WP. Development of GnRH antagonists for prostate cancer: New approaches to treatment. *Oncologist* 2000;5:162–168.
33. Wild JS, Hyde DM, Hubbell HR, Giri SN. Dose-related effects of Ampligen (poly(I).poly(C12U)), a mismatched double-stranded RNA, in a bleomycin-mouse model of pulmonary fibrosis. *Exp Lung Res* 1996;22: 375–391.
34. Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG. Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy. *Crit Rev Oncol Hematol* 2001;38:17–23.
35. Chirigos MA, Talor E, Sidwell RW, Burger RA, Warren RP. Leukocyte Interleukin, Inj. (LI) augmentation of natural killer cells and cytolytic T-lymphocytes. *Immunopharmacol Immunotoxicol* 1995;17:247–264.
36. Weiden PL, Breitz HB. Pretargeted radioimmunotherapy (PRIT) for treatment of non-Hodgkin's lymphoma (NHL). *Crit Rev Oncol Hematol* 2001;40:37–51.
37. Silver MA, Horton DP, Ghali JK, Elkayam U. Effect of nesiritide versus dobutamine on short-term outcomes in the treatment of patients with acutely decompensated heart failure. *J Am Coll Cardiol* 2002;39:798–803.
38. Colucci WS, Elkayam U, Horton DP, Abraham WT, Bourge RC, Johnson AD, et al. Intravenous nesiritide, a natriuretic peptide, in the treatment of decompensated congestive heart failure: Nesiritide Study Group. *N Engl J Med* 2000;343:246–253.
39. da Silva AJ, Brickelmaier M, Majeau GR, Li Z, Su L, Hsu YM, et al. Alefacept, an immunomodulatory recombinant LFA-3/IgG1 fusion protein, induces CD16 signaling and CD2/CD16-dependent apoptosis of $CD^{2+}$ cells. *J Immunol* 2002;168:4462–4471.
40. Ellis CN, Krueger GG. Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. *N Engl J Med* 2001; 345:248–255.
41. Deeg HJ, Blazar BR, Bolwell BJ, Long GD, Schuening F, Cunningham J, et al. Treatment of steroid-refractory acute graft-versus-host disease with anti-CD147 monoclonal antibody ABX-CBL. *Blood* 2001;98:2052–2058.
42. Piascik P. Omalizumab: A novel monoclonal antibody for treatment of allergic disease. *J Am Pharm Assoc (Wash)* 2002;42:356–358.
43. Leonard JP, Link BK. Immunotherapy of non-Hodgkin's lymphoma with hLL2 (epratuzumab, an anti-CD22 monoclonal antibody) and Hu1D10 (apolizumab). *Semin Oncol* 2002;29:81–86.
44. Harwood SJ, Gibbons LK, Goldner PJ, Webster WB, Carroll RG. Outpatient radioimmunotherapy with Bexxar: Closed, clean air reservoir minimizes personnel radiation exposure. *Cancer* 2002;94(4 Suppl): 1358–1362.
45. Gordon FH, Lai CW, Hamilton MI, Allison MC, Srivastava ED, Fouweather MG, et al. A randomized placebo-controlled trial of a humanized monoclonal antibody to $\alpha$4 integrin in active Crohn's disease. *Gastroenterology* 2001;121:268–274.
46. Sheremata WA, Vollmer TL, Stone LA, Willmer-Hulme AJ, Koller M. A safety and pharmacokinetic study of intravenous natalizumab in patients with MS. *Neurology* 1999;52:1072–1074.
47. Linn JF, Black P, Derksen K, Rubben H, Thuroff JW. Keyhole limpet haemocyanin in experimental bladder cancer: Literature review and own results. *Eur Urol* 2000;37(Suppl 3):34–40.
48. McNeil C. Monoclonal antibodies progress to the next generation. *J Natl Cancer Inst* 1995;87:1738–1739.
49. Breedveld FC. Therapeutic monoclonal antibodies. *Lancet* 2000;355: 735–740.
50. King KM, Younes A. Rituximab: Review and clinical applications focusing on non-Hodgkin's lymphoma. *Expert Rev Anticancer Ther* 2001; 1:177–186.
51. Grillo-Lopez AJ, Hedrick E, Rashford M, Benyunes M. Rituximab: Ongoing and future clinical development. *Semin Oncol* 2002;29(1 Suppl 2):105–112.
52. Bondeson J, Maini RN. Tumour necrosis factor as a therapeutic target in rheumatoid arthritis and other chronic inflammatory diseases: The clinical experience with infliximab (REMICADE). *Int J Clin Pract* 2001; 55:211–216.
53. Ricart E, Panaccione R, Loftus EV, Tremaine WJ, Sandborn WJ. Infliximab for Crohn's disease in clinical practice at the Mayo Clinic: The first 100 patients. *Am J Gastroenterol* 2001;96:722–729.
54. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; 344:783–792.
55. Pastore RD, Pfeffer LM, Nanus DM. Renal cell carcinoma and interferon at the millennium. *Cancer Invest* 2001;19:281–291.
56. Parmiani G, Rivoltini L, Andreola G, Carrabba M. Cytokines in cancer therapy. *Immunol Lett* 2000;74:41–44.
57. Zanussi S, De Paoli P. The effects of interleukin-2 therapy on the viral reservoir in HIV+ patients. *Biomed Pharmacother* 2000;54:316–320.
58. Flaherty LE, Atkins M, Sosman J, Weiss G, Clark JI, Margolin K, et al. Outpatient biochemotherapy with interleukin-2 and interferon $\alpha$-2b in patients with metastatic malignant melanoma: Results of two Phase II cytokine working group trials. *J Clin Oncol* 2001;19:3194–3202.
59. Ahmed A, Keeffe EB. Hepatitis C virus and liver transplantation. *Clin Liver Dis* 2001;5:1073–1090.
60. Haas GP, Hillman GG. Update on the role of immunotherapy in the management of kidney cancer. *Cancer Control* 1996;3:536–541.
61. Dempke W, Von Poblozki A, Grothey A, Schmoll HJ. Human hematopoietic growth factors: Old lessons and new perspectives. *Anticancer Res* 2000;20:5155–5164.
62. Duhrsen U. The clinical value of erythropoietin in patients with cancer. *Drugs* 2002;62:2013–2023.
63. Tong EM, Nissenson AR. Erythropoietin and anemia. *Semin Nephrol* 2001;21:190–203.
64. Anderson WF. Human gene therapy. *Nature* 1998;392:25–30.
65. Galanis E, Vile R, Russell SJ. Delivery systems intended for in vivo gene therapy of cancer: Targeting and replication competent viral vectors. *Crit Rev Oncol Hematol* 2001;38:177–192.
66. Wilson JM. Vectors: Shuttle vehicles for gene therapy. *Clin Exp Immunol* 1997;107(Suppl 1):31–32.
67. Makin G, Hickman JA. Apoptosis and cancer chemotherapy. *Cell Tissue Res* 2000;301:143–152.
68. Moul JW. Angiogenesis, p53, bcl-2 and Ki-67 in the progression of prostate cancer after radical prostatectomy. *Eur Urol* 1999;35:399–407.
69. Nielsen LL, Maneval DC. P53 tumor suppressor gene therapy for cancer. *Cancer Gene Ther* 1998;5:52–63.
70. Jaffee EM. Immunotherapy of cancer. *Ann N Y Acad Sci* 1999;886:67–72.
71. Bocchia M, Bronte V, Colombo MP, De Vincentiis A, Di Nicola M, Forni G, et al. Antitumor vaccination: Where we stand. *Haematologica* 2000;85:1172–1206.
72. Ries S, Korn WM. ONYX-015: Mechanisms of action and clinical potential of a replication-selective adenovirus. *Br J Cancer* 2002;86:5–11.
73. Nemunaitis J, Khuri F, Ganly I, Arseneau J, Posner M, Vokes E, et al.

Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J Clin Oncol* 2001;19:289–298.

74. Sasaki R, Shirakawa T, Zhang ZJ, Tamekane A, Matsumoto A, Sugimura K, et al. Additional gene therapy with Ad5CMV-p53 enhanced the efficacy of radiotherapy in human prostate cancer cells. *Int J Radiat Oncol Biol Phys* 2001;51:1336–1345.
75. Wada Y, Gotoh A, Shirakawa T, Hamada K, Kamidono S. Gene therapy for bladder cancer using adenoviral vector. *Mol Urol* 2001;5:47–52.
76. McNeel DG, Disis ML. Tumor vaccines for the management of prostate cancer. *Arch Immunol Ther Exp* 2000;48:85–93.
77. Hoffman DM, Figlin RA. Intratumoral interleukin 2 for renal-cell carcinoma by direct gene transfer of a plasmid DNA/DMRIE/DOPE lipid complex. *World J Urol* 2000;18:152–156.
78. Cancer vaccine: Antigenics. *BioDrugs* 2002;16:72–74.
79. Banerjee D. Genasense (Genta Inc.). *Curr Opin Investig Drugs* 2001;2:574–580.
80. Small EJ, Fratesi P, Reese DM, Strang G, Laus R, Peshwa MV, et al. Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. *J Clin Oncol* 2000;18:3894–3903.
81. Naitoh J, Witte O, Belldegrun A. The University of California, Los Angeles/Jennifer Jones Simon Foundation symposium on prostate cancer and epithelial cell biology: Bringing together basic scientists and clinicians in the fight against advanced prostate cancer. *Cancer Res* 1998;58:2895–2900.
82. O'Rourke MG, Schmidt CW, O'Rourke TR, Ellem KA. Immunotherapy, including gene therapy, for metastatic melanoma. *Aust N Z J Surg* 1997;67:834–841.
83. Daja MM, Aghmesheh M, Ow KT, Rohde PR, Barrow KD, Russell PJ. $\beta$-human chorionic gonadotropin in semen: A marker for early detection of prostate cancer? *Mol Urol* 2000;4:421–427.
84. Sanda MG, Smith DC, Charles LG, Hwang C, Pienta KJ, Schlom J, et al. Recombinant vaccinia-PSA (PROSTVAC) can induce a prostate-specific immune response in androgen-modulated human prostate cancer. *Urology* 1999;53:260–266.
85. Chen Y, Yu DC, Charlton D, Henderson DR. Pre-existent adenovirus antibody inhibits systemic toxicity and antitumor activity of CN706 in the nude mouse LNCaP xenograft model: Implications and proposals for human therapy. *Hum Gene Ther* 2000;11:1553–1567.
86. Yu DC, Chen Y, Dilley J, Li Y, Embry M, Zhang H, et al. Antitumor synergy of CV787, a prostate cancer-specific adenovirus, and paclitaxel and docetaxel. *Cancer Res* 2001;61:517–525.
87. Scholl SM, Balloul JM, Le Goc G, Bizouarne N, Schatz C, Kieny MP, et al. Recombinant vaccinia virus encoding human MUC1 and IL2 as immunotherapy in patients with breast cancer. *J Immunother* 2000;23:570–580.
88. Sweeney P, Pisters LL. Ad5CMVp53 gene therapy for locally advanced prostate cancer: Where do we stand? *World J Urol* 2000;18:121–124.
89. Lodge PA, Jones LA, Bader RA, Murphy GP, Salgaller ML. Dendritic cell-based immunotherapy of prostate cancer: Immune monitoring of a phase II clinical trial. *Cancer Res* 2000;60:829–833.
90. Geary RS, Henry SP, Grillone LR. Fomivirsen: Clinical pharmacology and potential drug interactions. *Clin Pharmacokinet* 2002;41:255–260.
91. Jabs DA, Griffiths PD. Fomivirsen for the treatment of cytomegalovirus retinitis. *Am J Ophthalmol* 2002;133:552–556.
92. Weng DE, Usman N. Angiozyme: A novel angiogenesis inhibitor. *Curr Oncol Rep* 2001;3:141–146.
93. Biederer C, Ries S, Brandts CH, McCormick F. Replication-selective viruses for cancer therapy. *J Mol Med* 2002;80:163–175.
94. Murakami P, Pungor E, Files J, Do L, van Rijnsoever R, Vogels R, et al. A single short stretch of homology between adenoviral vector and packaging cell line can give rise to cytopathic effect-inducing, helper-dependent E1-positive particles. *Hum Gene Ther* 2002;13:909–920.
95. Askari FK, McDonnell WM. Antisense-oligonucleotide therapy. *N Engl J Med* 1996;334:316–318.

*Better to be kind at home*
*Than to burn incense in a distant place.*

—Chinese proverb