27. J. M. Kerr, S. C. Banville, R. N. Zuckermann, *J. Am. Chem. Soc.* **115**, 2529 (1993).
28. V. Nikolaiev *et al.*, *Peptide Res.* **6**, 161 (1993).
29. M. H. J. Ohlmeyer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10922 (1993).
30. P. G. Komarov *et al.*, *Science* **285**, 1733 (1999).
31. T. U. Mayer *et al.*, *Science* **286**, 971(1999).
32. M. R. Spaller, M. T. Burger, M. Fardis, P. A. Bartlett, *Curr. Opin. Chem. Biol.* **1**, 47 (1997).
33. D. G. Powers *et al.*, *Tetrahedron* **54**, 4085 (1998).
34. D. A. Heerding, D. T. Takata, C. Kwon, W. F. Huffman, J. Samanen, *Tetrahedron Lett.* **39**, 6815 (1998).
35. S. H. Bertz, *J. Am. Chem. Soc.* **104**, 5801 (1982).
36. A. de Meijere *et al.*, *Angew. Chem. Int. Ed. Engl.* **38**, 3669 (1999).
37. P. Brooking *et al.*, *Synthesis* **1999**, 1986 (1999).
38. S. C. Schürer and S. Blechert, *Synlett* **12**, 1879 (1999).
39. K. Paulvannan, *Tetrahedron Lett.* **40**, 1851 (1999).
40. D. Lee, J. K. Sello, S. L. Schreiber, *Org. Lett.*, **2**, 709 (2000).
41. W. J. Zuercher, M. Hashimoto, R. H. Grubbs, *J. Am. Chem. Soc.* **118**, 6634 (1996).
42. D. Lee, J. Sello, S. L. Schreiber, *J. Am. Chem. Soc.* **121**, 10648 (1999).
43. For an animation, see www-schreiber.chem.harvard.edu/home/animation.html.
44. J. M. Blaney and E. J. Martin, *Curr. Opin. Chem. Biol.* **1**, 54 (1997).
45. Colleagues at Harvard University's Department of Chemistry and Chemical Biology and Institute of Chemistry and Cell Biology, especially M. D. Shair and E. J. Corey, are thanked for their insights and suggestions. The National Institute of General Medical Sciences, the National Cancer Institute, and Merck and Co. are gratefully acknowledged for their support of organic synthesis relevant to drug discovery.

REVIEW

Mechanism-Based Target Identification and Drug Discovery in Cancer Research

Jackson B. Gibbs

Cancer as a disease in the human population is becoming a larger health problem, and the medicines used as treatments have clear limitations. In the past 20 years, there has been a tremendous increase in our knowledge of the molecular mechanisms and pathophysiology of human cancer. Many of these mechanisms have been exploited as new targets for drug development in the hope that they will have greater antitumor activity with less toxicity to the patient than is seen with currently used medicines. The fruition of these efforts in the clinic is just now being realized with a few encouraging results.

In some areas of the world, cancer has become or shortly will become the leading disease-related cause of death of the human population. For example, in the United States, cancer is the second leading cause of death behind cardiovascular disease, and it is projected that cancer will become the leading cause of death within a few years. There are two main reasons for this change. First, cancer is a disease of multiple accumulating mutations that are becoming manifest in human populations, which have enjoyed an increasingly prolonged life-span (*1*). Second, cardiovascular-related deaths are decreasing as a result of an increased understanding of the mechanisms underlying the disease, the identification of risk factors, which indicate life-style changes that can reduce the onset of disease, and the development of targeted molecular therapies. In contrast, the medical treatment of cancer still has many unmet needs. The main curative therapies for cancer—surgery and radiation—are generally only successful if the cancer is found at an early localized stage. Once the disease has progressed to locally advanced cancer or metastatic cancer, these therapies are less successful. Existing chemotherapeutic treatments are largely palliative in these advanced tumors, particularly in the case of the common epithelial tumors such as lung, colorectal, breast, prostate, and pancreatic cancers (*2*). Sometimes, sound mechanistically based chemotherapies are effective but only for a defined period of time. For example, antihormonal treatments of prostate cancer can initially shrink tumors but eventually fail when the residual tumor cells become hormone-independent. Although a few chemotherapeutic regimens have yielded lasting remissions or cures (for example, in testicular cancer and childhood leukemias), it is clear that new therapeutic options are necessary.

In the development of new chemotherapeutic agents, several issues need to be addressed, including improved and durable antitumor efficacy, reduction of toxicities, which can prevent effective dosing of potentially efficacious drugs, and prevention of drug resistance caused by the inherent genomic instability of tumors. Upon the discovery some 20 years ago of the first oncogene defects in cancer (*3*), it was envisioned that the genetic information could be translated into therapeutics that could selectively ablate tumors without the systemic side effects often associated with cancer drugs. The translation of that scientific information into potential new medicines is now starting to emerge. In looking ahead at new targets and new approaches to cancer drug discovery, it can be useful to look at which pharmacological treatments have worked in other diseases, such as cardiovascular disease, and over which time frame these developments occurred.

Medicines to treat hypertension evolved over a 40-year period (*4*). In the 1950s and 1960s, the drugs of choice included reserpine and methyldopa, both of which act in the central nervous system. An understanding of receptor pharmacology led to development of peripherally acting adrenergic receptor antagonists in the 1970s, and this evolved in the 1980s and 1990s to peripherally acting non-adrenergic agents, such as inhibitors of angiotensin-converting enzyme and angiotensin-receptor antagonists, which have far fewer side effects than the early centrally acting agents. The lessons to be learned here are that basic research discoveries on the fundamental mechanisms responsible for a disease state often lead to the most direct pharmaceutical approaches to manage the disease. However, successful treatments emerge from an iterative process that depends not only on the scientific learning curve but also on feedback from clinical trials where we learn whether our mechanistic ideas are having a therapeutic benefit and what the drawbacks are in terms of side effects. The development of initial drugs and subsequent pharmacological improvements also benefits from knowledge of the specific molecular target of the drug, such as a receptor or enzyme. It takes decades to learn what approaches can initially provide some benefit for a disease and to then progress to a point where the disease is effectively managed with medicines essentially devoid of side effects.

Where Are We in Cancer?

Cancer chemotherapy emerged in the 1940s from toxicological studies of nitrogen mustard–based war gas (*2*). The anticancer activity of nitrogen mustard is due to DNA alkylation, and many other cancer drugs were developed on the basis of this general concept (modification of DNA, which impairs accurate replication) and then optimized on the basis of cytotoxicity in growth proliferation models. Mechanism-based approaches have also been explored for several decades. Antimetabolite drugs (for example, methotrexate and mercaptopurine) were developed on

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the basis of a scientific understanding of key enzyme steps in nucleotide biosynthesis and the sensitivity of tumor cells to alterations in these pathways. Newer agents in this class, such as gemcitabine (Gemzar), continue to show promise in the management of some cancers. Overall, though, the identification of successful agents with clinical utility has been a somewhat empirical process up to now, not least because of the focus on the antitumor efficacy of potential new agents in cell-culture cytotoxicity assays and animal tumor models that do not effectively reflect the complexities of human cancer. This often makes it difficult to assess why a particular drug can be successful (as cisplatin is for testicular cancer) or why others may fail. In the absence of a specific mechanistic understanding, particularly with regard to a molecular target, it is difficult to learn from the successes and failures and understand why different tumor types have different susceptibilities.

Over the last 20 years, there has been a fundamental shift in the way target identification in cancer is approached. Advances in molecular biology now allow us to identify genes that go awry in cancer, and offer the opportunity to dissect the molecular mechanisms underlying the disease. At first, there were just a handful of cancer genes (such as *src*, *abl*, *ras*, *myc*, *myb*, *mos*, and *raf*), and the challenge was to find out how the gene products functioned. Now, many genes are known to affect tumorigenesis and tumor growth (some are shown in Fig. 1), and the key is to decide which ones to exploit in the areas of signal transduction, cell-cycle regulation, apoptosis, telomere biology, and angiogenesis (*5*).

Cancer Target Identification and Pharmaceutical Tractability

Nothing provides more compelling validation for a target than knowledge of the human genetics of a specific disease. In cancer research, the choice of target is often highlighted by the mutated gene underlying the cancer (such as *ras*, *p53*, *RB*, *p16*, *myc*, and *bcr-abl* shown in Fig. 1) (*3, 6*). Overexpression of specific gene products, such as HER-2, epidermal growth factor (EGF) and insulin-like growth factor receptors, and cyclins, has also been correlated as a causative factor in some cancers (*6–8*). Alternatively, a normal gene product may be closely correlated with cancer progression. For example, elevated telomerase activity is observed in essentially all human cancers (*9, 10*), and increased serum vascular endothelial growth factor (VEGF) has been reported to be a prognostic clinical factor correlated with decreased survival in breast, ovarian, lung, gastric, and colon cancer patients (*11*). Obviously, many molecular tools are available for target validation, including antisense oligonucleotides, ribozymes, dominant negative mutants, neutralizing antibodies, and mouse transgenics/knockouts. Often multiple approaches must be evaluated. For example, the use of several of these tools has led to the recognition that the telomerase enzyme (*9, 10, 12*) and the KDR receptor of VEGF (*11, 13*) are good targets for drug development. Telomerase regulates the immortalization properties of tumors and KDR is expressed in normal vasculature endothelial cells that wc ' ' not be expected to have the genomic instal _ / problems of the surrounding tumor cells.

A genetic defect in a tumor may identify a potential target, but it will never serve as a successful target for drug discovery unless it is pharmaceutically tractable. This is often frustrating, because many more targets can be validated by the tools of molecular biology than may be amenable for drug development. For example, there is strong evidence that if one were to disrupt protein-protein interactions such as Myc/Max dimerization, specific SH2 domain interactions, or Ras/Raf binding, one could inhibit the function of pathways essential to certain tumor cells (*14, 15*). Progress to develop inhibitors of these protein-protein interactions has, however, proven problematic. Model peptides have been found, but they are often difficult to convert into molecules with appropriate pharmaceutical properties (*14, 15*). This process often entails the development of low molecular weight (<600 daltons) organic molecules with sufficient potency, pharmacokinetic, and safety profiles to be considered a drug suitable for testing in humans. A notable example where inhibitors of protein-protein interaction have been developed is in the area of integrin biology, where antagonists of specific integrins may serve as anti-angiogenesis agents (*16, 17*). Newer approaches such as therapeutic antibodies and nucleic acid–based treatments (antisense oligonucleotides) may afford alternative ways to attack targets that are not amenable to small-molecule inhibitor development, as will be discussed later.

A knowledge of the cellular signaling pathways can also be helpful for exploiting rational targets that prove difficult to inhibit, either for the reasons described above or because, in the case of tumor suppressor genes, the protein target is no longer present in the tumor. For example, many of the early approaches to inhibit Ras function failed but knowledge of the pathways afforded new targets in Raf and MEK (*18*). Likewise, inhibition of specific cyclin dependent kinases by *p16* indicates that protein kinase inhibitors to these targets may inhibit tumors having defective *p16* (*7, 8*). Alternatively, there may be new approaches to find pharmaceutically viable targets that might not otherwise be identified by our current knowledge of cancer genetics. For example, yeast genetic screens were developed to identify which other pro-

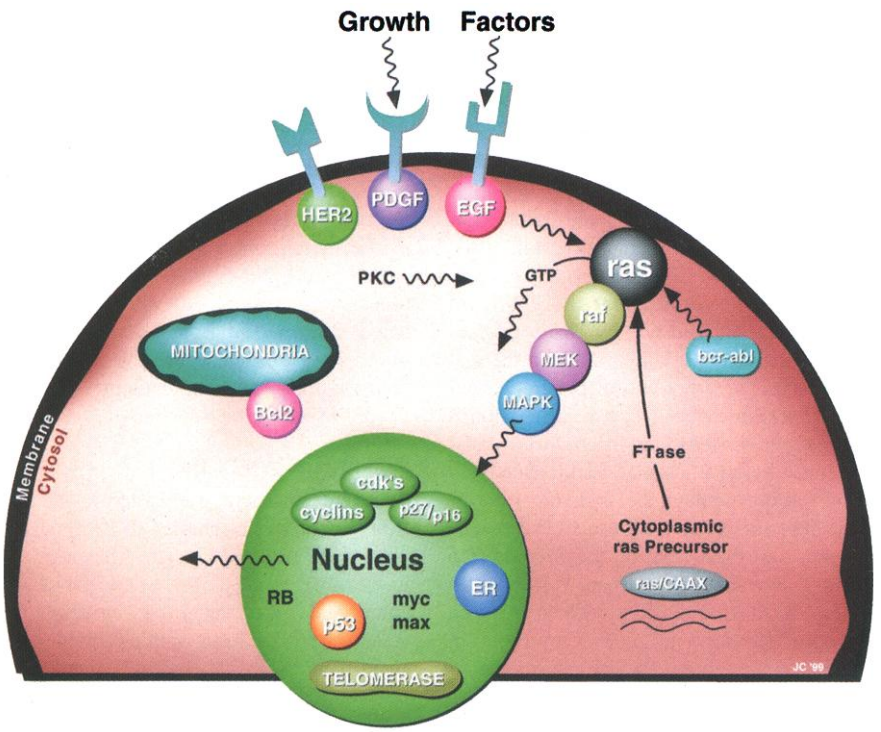

Fig. 1. Examples of molecular targets in tumor cells for cancer drug development. See text for details. cdk's, cyclin-dependent kinases; EGF, epidermal growth factor receptor; ER, estrogen receptor; FTase, farnesyltransferase; MEK, MAPK/Erk kinase; PDGF, platelet-derived growth factor receptor; PKC, protein kinase C.

tein(s) when inhibited selectively kill cells that have the primary gene defect commonly found in human cancers (*19*). This strategy used to identify "secondary" targets is known as synthetic lethality. Gene chip technologies also afford ways to elucidate critical pathways that might serve to identify targets for inhibitor development (*20, 21*).

Breast Cancer as a Paradigm

In the development of new cancer therapeutics, breast cancer can serve as a useful example for the application of both traditional cytotoxic approaches and new mechanistic information. The standard of care often includes doxorubicin (Adriamycin) and a taxane such as paclitaxel (Taxol) or docetaxel (Taxotere) (*2*). Recent advances have been realized with the emergence of tamoxifen (Nolvadex) and trastuzumab (Herceptin), which were developed on the basis of insights into the role of estrogen in breast cancer and oncogenes, respectively.

Although doxorubicin and the taxanes were discovered on the basis of their toxicity to tumor cells in preclinical models, subsequent studies identified the biochemical mechanisms of action. Doxorubicin inhibits topoisomerase functions, and more specific topoisomerase inhibitors have been subsequently developed. Taxanes stimulate tubulin polymerization and induce tumor apoptosis via a novel G_2/M checkpoint that is independent of wild-type *p53* function (*22, 23*). Taxanes achieve responses in 30 to 40% of patients, and this level of clinical success has sparked further preclinical efforts in this area (*24*). For example, novel agents such as the epothilones were identified in tubulin polymerization screens and have been shown to be active in preclinical models of cancers that are resistant to paclitaxel (*25*). A cell-based assay, which looks at mitotic spindle organization, has also been designed to identify novel agents that might modulate the G_2/M checkpoint in a less-toxic manner than the taxanes (*26*).

The role of estrogen in some breast cancers was recognized as early as the 1930s, but the field really exploded with the identification of the first estrogen receptor in the 1960s and the realization that measuring estrogen receptor levels in clinical breast cancer specimens could prospectively identify hormone-dependent tumors (about 50% of all breast cancer cases) that might be responsive to endocrine therapy (*27, 28*). These studies ultimately led to the development of tamoxifen, an antagonist of the estrogen receptor in breast tissue, for adjuvant therapy of breast cancer. Tamoxifen reduces the incidence of cancer recurrence in patients with estrogen receptor positive tumors. It can also delay the occurrence of breast cancer in women who are at high risk for developing the disease. These benefits are realized with minimal drug-related toxicity. Nevertheless, resistance to tamoxifen inevitably occurs. Efforts to improve on tamoxifen will likely benefit from the identification of a second estrogen receptor (ER-β) and research in the area of selective estrogen receptor modulators (SERMs) (*28, 29*), which can have agonist activity in one tissue and antagonist activity in another tissue. Tamoxifen has demonstrated activity to prevent cancer because of its antagonist properties in breast tissue but its use is currently limited to cancer patients because of some of its mechanism-based side effects, such as a slightly increased risk for endometrial cancer, which is due to the agonist activity of tamoxifen in the uterus (*28*). In addition to newer SERMs that are active against tamoxifen-resistant tumors, an ultimate goal will be the development of effective estrogen agonist hormone replacement therapies (for bone, lipids, and hot flashes in post-menopausal women) that can also be used to prevent cancers because of estrogen antagonist activity in breast and uterine tissues (*28, 29*). The development of next generation SERMs is a highly active area of research (Table 1).

Trastuzumab serves as the first success story for therapeutic strategies based on studies of oncogenes (*30*). The HER-2 gene was discovered in 1985, and the overexpression of its gene product was subsequently associated with human breast cancer in 1987 (*6, 30*). Genentech pursued the development of an antibody directed to the extracellular domain of HER-2. The rationale was to develop an agent that will selectively recognize tumor cells having a defective gene product. Trastuzumab had an excellent preclinical antitumor profile, particularly when used in combination with a second agent such as doxorubicin or paclitaxel (*31*). The therapeutic antibody appears to work by several mechanisms including internalization and down-regulation of HER-2 receptor, induction of cell-cycle inhibitors such as p27kip, and immune-mediated responses (*32*). Trastuzumab is well tolerated by patients, and the drug was approved by the U.S. Federal Drug Administration (FDA) in 1998 for the treatment of HER-2–positive breast cancer (which accounts for approximately 30% of breast cancer). Responses of up to 60 to 70% have been noted for trastuzumab in combination with paclitaxel (*33*). Improved efficacy is also noted in patients treated with both trastuzumab and doxorubicin, but this combination also results in greater cardiotoxicity (*34*). This higher toxicity appears to be mechanism-based, because HER-2 is expressed in heart tissue and doxorubicin is known to be a cardiotoxic agent.

New Molecular-Based Agents in Clinical Trials

The translation of mechanism-based target identification to new therapies is now being realized on a large scale in the clinic. In particular, protein kinases have drawn special attention (Table 1). Some of the more promising clinical trials are being done with a small-molecule inhibitor of Bcr-abl and either small-molecule inhibitors or a therapeutic antibody of the EGF receptor.

The choice of Bcr-abl as a target is based on the Philadelphia chromosome, one of the first defined genetic alterations diagnostic for a cancer, chronic myelogenous leukemia (CML) (*35*). The fusion between the *bcr* and *abl* genes is the molecular basis of the defect because the resulting Bcr-abl gene product has an abnormal tyrosine kinase activity. CGP 57148/STI 571 is a potent inhibitor of both Bcr-abl and platelet-derived growth factor receptor protein kinase activities, and the inhibitor cures *bcr-abl* tumors in mice as long as the drug is administered so that Bcr-abl protein kinase activity is blocked continuously (*35*). In clinical trials, nearly all CML

Table 1. Examples of mechanism-based cancer therapies in development. See text and Fig. 1 for details.

Drug	Target
Receptor antagonists	
Raloxifene	Estrogen receptor
LY353381	Estrogen receptor
GW 5638	Estrogen receptor
CP336156	Estrogen receptor
EM-800	Estrogen receptor
EMD-121974	Integrin
Protein kinase inhibitors	
CGP 57148/STI 571	Bcr-abl
ZD-1839	EGF receptor
CP-358,774	EGF receptor
SU-5416	KDR
SU-6668	KDR, FGFR, and PDGFR
CGP 60474	Cyclin-dependent kinases
Flavopiridol	Cyclin-dependent kinases
CGP 41251	Protein kinase C
UCN-01	Protein kinase C
Other enzyme inhibitors	
R115777	FTase
SCH 66336	FTase
L-778,123	FTase
BMS-214662	FTase
CP-609,754	FTase
Marimastat	MMPs
AG-3340	MMPs
BMS-275291	MMPs
CGS-27023A	MMPs
BAY 12-9566	MMPs
Therapeutic antibodies	
C225	EGF receptor
Anti-VEGF	VEGF
IMC-1C11	KDR
Vitaxin	Integrin
Antisense oligonucleotides	
ISIS-2503	Ras
ISIS-5132	Raf
ISIS-3521	Protein kinase C
G3139	Bcl2
Viruses	
SCH 58500 (Ad-*p53*)	p53
Onyx-015	p53

patients who receive the appropriate dose-level of CGP 57248/STI 571 have shown complete responses, and these responses have been sustained for at least 8 months with minimal side effects (*35*).

EGF receptors are overexpressed in some tumors such as lung, oral, and colon cancer, and autocrine activation of this receptor by EGF and TGF-α is important to the proliferation of the tumor cells (*6*). The EGF receptor kinase inhibitors ZD-1839 and CP-358,774 also appear to be showing good clinical responses, particularly in non–small cell lung cancer (*36–38*). The EGF receptor kinase inhibitors are well-tolerated, and the dermatological toxicities reported to date are most likely mechanism-based.

Should the initial encouraging results with these small-molecule protein kinase inhibitors continue, it would reinforce efforts to develop agents directed at the primary defects in cancer. Furthermore, the results would support continued efforts to develop protein kinase inhibitors. Even though these agents are competitive with respect to adenosine triphosphate and might be expected to inhibit other protein kinases, they appear to have sufficient biochemical specificity to be well tolerated by the patients. Other protein kinase inhibitors in clinical trials (Table 1) also appear to be tolerated sufficiently to allow testing beyond phase I (*8, 16, 38, 39*).

Broad-based pharmaceutical efforts have also focused on developing inhibitors of farnesyltransferase (FTase) and matrix metalloproteinases (MMPs) (Table 1). FTase became a major target for inhibitor development after it was shown to catalyze an essential lipidation step onto the COOH-terminus (the CaaX sequence) of the *ras* oncogene proteins; without this lipidation, Ras proteins cannot transform normal cells into tumor cells (*38, 40–42*). However, at least 20 other proteins have been identified so far which are also substrates for FTase, indicating that FTase inhibitors (FTIs) cannot be considered Ras-specific drugs. FTIs have not demonstrated any strong antitumor activity in humans when tested as single agents in phase I studies, but several FTIs are now being tested clinically in combination with other therapies based on the enhanced antitumor activity seen in preclinical cancer studies (*38, 41*).

MMPs are involved with multiple aspects of tumor physiology including tumor cell metastasis and endothelial cell invasion essential to tumor angiogenesis (*43*). Given that there are more than 15 different MMPs, a key question is which MMPs one should inhibit. The MMP inhibitors currently in clinical trials have different specificities for the various MMPs, and the results of these trials will help in identifying the most appropriate biochemical profile.

Several biological approaches to mechanistically validated targets are showing promise in clinical trials (Table 1). The therapeutic antibody C225 (made to the extracellular domain of the EGF receptor), in particular, is achieving complete responses in head and neck cancer when administered in combination with radiation (*44*). Therapeutic antibodies are also being evaluated as antitumor agents against the angiogenesis targets VEGF, KDR, and an integrin (*45*). Promising results are being reported for antisense oligonucleotides directed at key regulatory proteins in tumor cells, and may offer methods to inhibit enzymes that are difficult to specifically inhibit biochemically (*46, 47*). Antisense oligonucleotides also offer a means to attack proteins that are not amenable to small-molecule inhibitor development. The up-regulation of the anti-apoptotic protein Bcl2 is a very common alteration in human cancer and is associated with resistance to chemotherapeutics and radiation (*48*). Inhibition of Bcl2 dimerization with pro-apoptotic partners has so far proven difficult to disrupt chemically. G3139 appears to effectively lower *bcl2* RNA expression in tissues, and clinical tumor responses have been reported with combination therapies (*47*).

Gene therapy has the potential to offer new biologically based medicines. Some gene therapy approaches have focused on the p53 protein, which until recently has not been amenable to small-molecule inhibitor development (*48–51*). In about 50% of tumors, the p53 protein is either lost or mutated such that it adopts a conformation that ablates its ability to bind DNA. Wild-type *p53* is often critical for an apoptotic response to DNA-damaging agents, and therefore, loss of *p53* function is a major cause of tumor resistance to chemotherapeutic agents (*48*). Clinical trials focused on *p53* are trying to either reintroduce wild-type *p53* back into tumor tissues or to inoculate the tumor with a cytolytic virus that replicates selectively in cells that have lost wild-type *p53* function (Table 1). A recent study has provided proof-of-concept that pharmacological approaches are also possible to restore function to mutant p53 proteins (*51*). In a screen that monitored the active and inactive conformations of p53, small molecules of 300 to 500 daltons were identified that could restore functional activity to p53 proteins having one of four different mutations. One of these compounds, CP-31398, significantly inhibited tumor growth in animals when it was administered at doses that restored wild-type function to mutant p53.

Conclusions

The promise of new molecularly based medicines founded on a genetic understanding of cancer is in the process of being realized, and the clinical results will point to new research directions. Some of the most exciting results are obtained with agents directed against tyrosine kinases, either as therapeutic antibodies or as small-molecule kinase inhibitors. However, the problem of tumor instability that might lead to resistance is a looming issue, and it still remains to be seen whether these new drugs will offer lasting survival advantages to the patients. It is also apparent that not all of the approaches are performing as well as anticipated. There is clearly a learning curve with respect to the best ways to use these new agents, just as has been the case in the development of traditional cytotoxic drugs. For example, many of the new agents are being tested in combination with therapies currently used to treat specific cancers. A number of toxicities are also encountered, some of which are mechanism-based (EGF receptor inhibitors, FTIs, MMPIs) and some of which are caused by the chemical structure of drug unrelated to its mechanism of action (phosphorothioate antisense oligonucleotides) (*38, 46*). Nevertheless, these newer agents are affording novel ways to mechanistically attack cancer, even if one cannot realize efficacy without toxicity.

An increased use of tumor genotyping to guide the choice of cancer therapy can also be anticipated (*1*), as is already being done in breast cancer to determine estrogen receptor and HER-2 status. The ultimate goal is still to obtain the best agents that will achieve complete and durable responses. Targeting the most appropriate patients may be a way of using newer medicines in the most effective manner and achieve a therapeutic index (a ratio between the dose that achieves an antitumor effect versus the dose that gives toxicity) that is greater than currently obtainable in the clinic with cytotoxic agents. This promise is being realized in current clinical trials with the Bcr-abl protein kinase inhibitor. One can also envision highly safe drugs that can prevent cancer, as is being anticipated with newer SERMs for breast cancer, 5α-reductase inhibitors for prostate cancer, and inhibitors of cyclo-oxygenase 2 for colon polyps and potentially colon cancer (*52*). Each step in this slow and sometimes stochastic process, however, should benefit from rigorous target validation and novel mechanistic approaches to enhance the cancer drug development process.

References and Notes

1. W. G. Kaelin Jr., *J. Clin. Invest.* **104**, 1503 (1999).
2. B. A. Chabner, C. J. Allegra, G. A. Curt, P. Calabresi, in *Goodman and Gilman's the Pharmacological Basis of Therapeutics*, J. G. Hardman, A. G. Gilman, L. E. Limbird, Eds. (McGraw-Hill, New York, 1996), pp. 1233–1287.
3. J. M. Bishop, *Cell* **64**, 235 (1991).
4. J. A. Oates, in (*2*), pp. 780–808.
5. D. Hanahan and R. A. Weinberg, *Cell* **100**, 57 (2000).
6. A. S. Perkins and D. F. Stern, in *Cancer: Principles and Practice of Oncology*, V. T. DeVita, S. Hellman, S. A. Rosenberg, Eds. (Lippincott-Raven, Philadelphia, 1997), pp. 79–102.

7. C. J. Sherr, *Science* **274**, 1672 (1996).
8. G. I. Shapiro and J. W. Harper, *J. Clin. Invest.* **104**, 1645 (1999).
9. W. C. Hahn *et al.*, *Nature Med.* **5**, 1164 (1999).
10. B. S. Herbert *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14276 (1999).
11. G. Neufeld, T. Cohen, S. Gengrinovitch, Z. Poltorak, *FASEB J.* **13**, 9 (1999).
12. T. de Lange and T. Jacks, *Cell* **98**, 273 (1999).
13. J. Holash, S. J. Wiegand, G. D. Yancopoulos, *Oncogene* **18**, 5356 (1999).
14. J. B. Gibbs and A. Oliff, *Cell* **79**, 193 (1994).
15. T. K. Sawyer, *Biopolymers* **47**, 243 (1998).
16. J. M. Hamby and H. D. H. Showalter, *Pharmacol. Ther.* **82**, 169 (1999).
17. B. P. Eliceiri and D. A. Cheresh, *J. Clin. Invest.* **103**, 1227 (1999).
18. D. C. Heimbrook, A. Oliff, J. B. Gibbs, in (*6*), pp. 35–45.
19. L. H. Hartwell, P. Szankasi, C. J. Roberts, A. W. Murray, S. H. Friend, *Science* **278**, 1064 (1997).
20. M. J. Marton *et al.*, *Nature Med.* **4**, 1293 (1998).
21. D. Fambrough, K. McClure, A. Kazlauskas, E. S. Lander, *Cell* **97**, 727 (1999).
22. J. S. Lanni, S. W. Lowe, E. J. Licitra, J. O. Liu, T. Jacks, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9679 (1997).
23. P. K. Sorger, M. Dobles, R. Tournebize, A. A. Hyman, *Curr. Opin. Cell Biol.* **9**, 807 (1997).
24. C. L. Vogel and J. M. Nabholtz, *Oncologist* **4**, 17 (1999).
25. I. Ojima *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 4256 (1999).
26. T. U. Mayer *et al.*, *Science* **286**, 971 (1999).
27. V. C. Jordan, *Trends Endocrinol. Metab.* **10**, 312 (1999).
28. A. S. Levenson and V. C. Jordan, *Eur. J. Cancer* **35**, 1628 (1999).
29. D. P. McDonnell, *Trends Endocrinol. Metab.* **10**, 301 (1999).
30. M. M. Goldenberg, *Clin. Ther.* **21**, 309 (1999).
31. J. Baselga, L. Norton, J. Albanell, Y. M. Kim, J. Mendelsohn, *Cancer Res.* **58**, 2825 (1998).
32. M. X. Sliwkowski *et al.*, *Semin. Oncol.* **26**, 60 (1999).
33. S. Shak, *Semin. Oncol.* **26**, 71 (1999).
34. M. S. Ewer, H. R. Gibbs, J. Swafford, R. S. Benjamin, *Semin. Oncol.* **26**, 96 (1999).
35. B. J. Druker and N. B. Lydon, *J. Clin. Invest.* **105**, 3 (2000).
36. J. D. Moyer *et al.*, *Cancer Res.* **57**, 4838 (1997).
37. D. H. Boschelli, *Drugs Future* **24**, 515 (1999).
38. J. B. Gibbs, *J. Clin. Invest.* **105**, 9 (2000).
39. A. Gescher, *Gen. Pharmacol.* **31**, 721 (1998).
40. A. Oliff, *Biochim. Biophys. Acta* **1423**, C19 (1999).
41. T. M. Williams, *Expert Opin. Ther. Patents* **9**, 1263 (1999).
42. D. M. Leonard and J. S. Sebolt-Leopold, *Drugs Future* **24**, 1099 (1999).
43. R. P. Beckett and M. Whittaker, *Experts Opin. Ther. Patents* **8**, 259 (1998).
44. Z. Fan and J. Mendelsohn, *Curr. Opin. Oncol.* **10**, 67 (1998).
45. E. Keshet and S. A. Ben-Sasson, *J. Clin. Invest.* **104**, 1497 (1999).
46. J. T. Holmlund, B. P. Monia, T. J. Kwoh, F. A. Dorr, *Curr. Opin. Mol. Ther.* **1**, 372 (1999).
47. F. E. Cotter, *Semin. Hematol.* **36**, 9 (1999).
48. W. R. Sellers and D. E. Fisher, *J. Clin. Invest.* **104**, 1655 (1999).
49. F. McCormick, *Cancer J. Sci. Am.* **5**, 139 (1999).
50. C. Heise *et al.*, *Nature Med.* **3**, 639 (1997).
51. B. A. Foster, H. A. Coffey, M. J. Morin, F. Rastinejad, *Science* **286**, 2507 (1999).
52. G. J. Kelloff, C. C. Sigman, P. Greenwald, *Eur. J. Cancer* **35**, 1755 (1999).

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REVIEW

Harnessing the Power of the Genome in the Search for New Antibiotics

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Over the past 40 years, the search for new antibiotics has been largely restricted to well-known compound classes active against a standard set of drug targets. Although many effective compounds have been discovered, insufficient chemical variability has been generated to prevent a serious escalation in clinical resistance. Recent advances in genomics have provided an opportunity to expand the range of potential drug targets and have facilitated a fundamental shift from direct antimicrobial screening programs toward rational target-based strategies. The application of genome-based technologies such as expression profiling and proteomics will lead to further changes in the drug discovery paradigm by combining the strengths and advantages of both screening strategies in a single program.

The science of genomics has largely been driven by the desire to understand the organization and function of the human genome. However, determination and characterization of smaller, less complex genomes, notably bacteria and yeast, has preceded that of the human genome, providing a testing ground for high-throughout screening procedures. For example, the *Saccharomyces cerevisiae* genome project, which delivered the first complete eukaryotic genome with 16 chromosomes and about 6200 genes (*1*), provides a model for ways in which DNA sequence information can be used to direct the subsequent systematic study of biochemical and functional processes (*2, 3*). Furthermore, new approaches are being developed for extracting information concerning gene expression, protein levels, subcellular localization and functionality (*4, 5*), providing for the first time a "genome view" of how an organism grows, reproduces, and responds to its environment.

Many of the complete genomes determined so far are of microorganisms, and further microbial genomes are being sequenced (Fig. 1). Microbial genomics are already revolutionizing the pharmaceutical industry's capability for antimicrobial drug hunting—and none too soon. Most antibiotic drugs used today are derivatives of agents which have been in the clinic for more than 30 years (or even longer in nature as natural products). This in itself would not be a problem, were it not for the remarkable ability of microorganisms to evolve and adapt. The biggest threat is antibiotic resistance (*6–8*). This has always been an issue, but in the early years of penicillin use pathogens depended on a single resistance mechanism, whereas many strains found in the clinic today have acquired multiple systems to reduce or avoid the action of an antibiotic (*9, 10*). Most threatening of these are the mechanisms that involve changes in the target site for antibiotic interaction, conferring levels of resistance to all compounds with that same mechanism of action. Furthermore, the DNA coding for these processes can be transferred between related strains, and the short generation time of many microorganisms facilitates the opportunity for gene selection even during a short course of drug treatment (*11*).

Resistance is not the only problem, however. As clinical practice changes to encompass greater use of invasive procedures and patients live longer, more and more individuals are becoming dependent on adequate antimicrobial cover. This is particularly relevant in the case of immunocompromised patients, who may be infected even by normally nonpathogenic organisms. Unfortunately, the use of antibiotics can select for such infections which are not sensitive to standard therapies. For example, in Europe, 10% of infections in intensive therapy units involve *Acinetobacter sp.* highly resistant but previously rare pathogens (*12*). In this way not only is resistance escalating, but also a new range of organisms have to be considered as potential pathogens.

There is therefore a need for a range of new drugs with new mechanisms of action, not susceptible to existing resistance mechanisms and in sufficient numbers to reduce reliance on a small number of chemical classes. Almost all antimicrobial compounds in the clinic today have come from semi-rational optimization programs based on compounds, often natural products, identified by whole-cell, antimicrobi-

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