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Perspective

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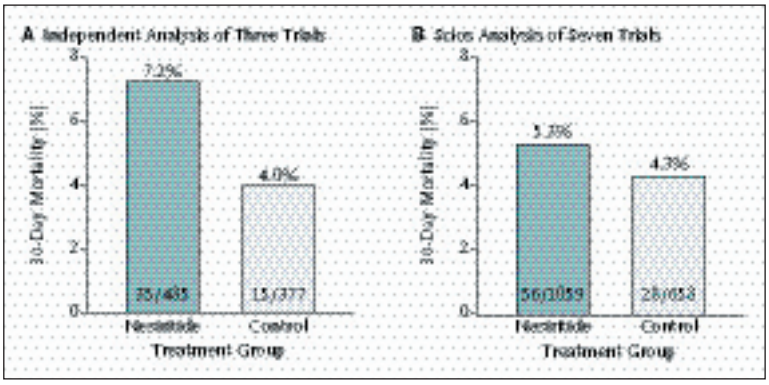
Nesiritide — Not Verified

Eric J. Topol, M.D.

How can a drug that is associated with higher rates of both renal dysfunction and death than placebo — and that costs 50 times as much as standard therapies and for which there are no meaningful data on relevant clinical end points — be given to more than 600,000 patients and be promoted throughout the United States for serial outpatient use, an indication not listed on the label?[1-5] The answer to this question can be discerned, at least in part, from a review of the clinical development and marketing of nesiritide: recombinant human brain natriuretic peptide.

On May, 17, 2005, the *New York Times* reported that tens of thousands of patients around the country are receiving nesiritide treatment once or more per week, over a period of several months, for what is described as an outpatient "tune-up." This application of nesiritide — which costs approximately $500 per dose, as compared with less than $10 for nitroglycerin or nitroprusside — is the subject of an aggressive marketing campaign by the manufacturer, Scios, which is encouraging physicians to start their own "infusion centers" for whose services they can bill Medicare as if they were providing chemotherapy. But when nesiritide was approved by the Food and Drug Administration (FDA) in 2001, it was designated for the treatment of acute, decompensated congestive heart failure. Moreover, it had apparent safety problems — and no proven clinical advantage over existing treatments in terms of the key end points of improved survival and prevention of subsequent hospitalizations.

In a 2000 report, Colucci and colleagues concluded that "nesiritide would be a valuable addition to the initial treatment of patients admitted to the hospital for decompensated congestive heart failure."[1] But their placebo-controlled, dose-ranging trial was focused on short-term monitoring of the pulmonary-capillary wedge pressure. Follow-up data on these subjects, which were not part of the study design as reported in that article, suggested that nesiritide may have had an adverse effect on 30-day mortality, which was 7.1 percent in the nesiritide group, as compared with 4.8 percent in the placebo group ($P=0.62$).[2] Over this longer period of follow-up, the incidence of substantial de-



Mortality at 30 Days among Patients Treated with Nesiritide as Compared with Controls in Randomized Trials.

The relative risk of death with nesiritide as compared with placebo was 1.81 (95 percent confidence interval, 1.02 to 3.27; P=0.04) in the analysis by Sackner-Bernstein et al.[3] (Panel A) and 1.26 (95 percent confidence interval, 0.80 to 2.00; P=0.33) in the analysis by Scios (Panel B).

terioration of renal function was more than three times as high among patients treated with nesiritide as among those given placebo (P=0.04).[3]

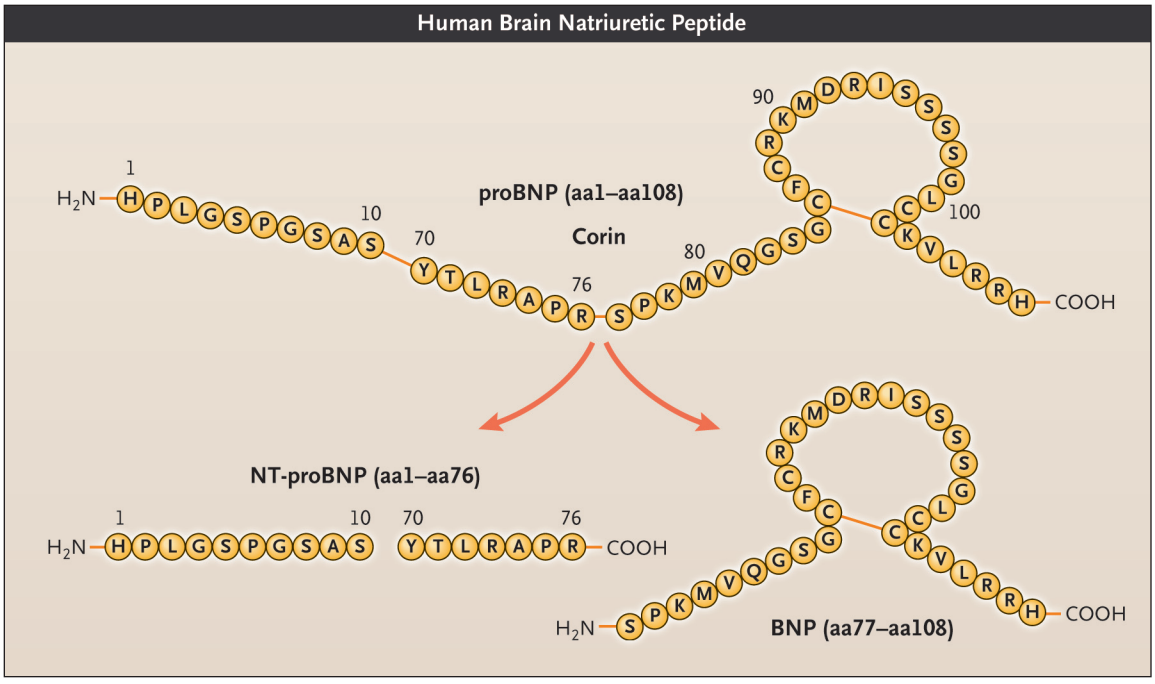
The data from that trial were reviewed by an FDA advisory panel in January 1999. Despite the panel's recommendation that the drug be approved for the reduction of pulmonary-capillary wedge pressure, the FDA decided that more data were required. A larger trial, Vasodilatation in the Management of Acute Congestive Heart Failure (VMAC), was conducted in 498 hospitalized patients who had dyspnea at rest.[4] Nesiritide was compared with intravenous nitroglycerin or placebo. Since there were statistically significant improvements in both the reduction of pulmonary-capillary wedge pressure (a difference of 4 mm Hg) and the self-reported dyspnea rating with nesiritide as compared with placebo, nesiritide was considered to have met the efficacy criteria. The study did not demonstrate any benefit of nesiritide over nitroglycerin in terms of death or the need for repeated hospitalization within 30 days. At a subsequent meeting, in May 2001, the majority of the advisory panel recommended granting approval for nesiritide, and in August 2001, the FDA formally approved this drug for commercial use.

The VMAC trial raised a number of concerns about nesiritide. Only 30 percent of the patients received furosemide or intravenous diuretics before enrollment, although use of these agents is a standard approach to acute decompensated heart failure. The dose of nitroglycerin was not titrated aggressively, and because of the monitoring of the pulmonary-capillary wedge pressure, the "double-blind" assessment was compromised. The length of stay in the hospital was greater among patients who received nesiritide than among those given nitroglycerin (10.0 vs. 8.1 days, P=0.008).[5] An elevation of more than 0.5 mg per deciliter (44.2 μmol per liter) in the serum creatinine level occurred in 27 percent of the patients in the nesiritide group, as compared with 21 percent of the controls (P=0.11).[3,4] There was no increase in urine output with nesiritide, and subsequent studies have shown that this drug does not have a natriuretic or diuretic effect in patients with decompensated heart failure. Furthermore, the rate of death at 30 days was 8.6 percent in the nesiritide group, as compared with 5.5 percent among the controls (relative risk, 1.56; 95 percent confidence interval, 0.75 to 3.24; P=0.20).[2] Indeed, the FDA approval went forward despite an internal reviewer's critical point that VMAC did not rule out a 50 percent increase in the risk of death. A meta-analysis also suggests that the use of nesiritide is associated with an increased frequency of abnormal renal function.[3]

There have been two different analyses of the effects of nesiritide treatment on mortality, the most important end point in a randomized trial of an intervention for heart failure (see graphs). In one of them, Sackner-Bernstein et al. pooled data from the three trials involving patients whose baseline treatment regimen was not required to include inotropes and for which 30-day mortality data were available. According to this analysis, there was an 81 percent increase in the death rate with nesiritide as compared with placebo.[2] In contrast, Scios analyzed seven trials that had 30-day mortality data, including trials involving open-label and outpatient use, but did not take into consideration the baseline treatment regimen. The company reported a 24 percent increase in mortality (P=0.33), and this figure was incorporated into a revised package insert in April 2005.

Even in the face of such find-

ings, however, the manufacturer has been actively promoting the use of nesiritide. It has set up a toll-free telephone hotline for "Natrecor Reimbursement Support" and has published a 46-page



"Natrecor Reimbursement and Billing Guide." The guide provides physicians with specific Medicare code numbers to be used in billing for a professional fee for nesiritide infusion ($172 for the first hour, $39 for each additional hour, and $408 for eight hours of observation), as one would for chemotherapy. The company justifies this billing practice by noting that the codes for chemotherapy administration include "substances such as monoclonal antibody agents and other biologic response modifiers."

Notwithstanding the fact that only one small, open-label feasibility study has been conducted, outpatient nesiritide use has become widespread, fulfilling sales objectives for the manufacturer and bringing in revenue for physicians. The overall sales figure for nesiritide is projected to be $700 million for 2005, nearly double last year's tally; it represents payment for more than 1.4 million treatments. Given that nearly 10 times as much drug is used for serial administration in outpatients as for the one-time use in hospitalized patients, much of this growth clearly stems from the off-label "tune-up" application.

The nesiritide story reflects some recurring themes: in other recent cases, too, major safety problems have been uncovered after a drug has been approved. Nesiritide was approved on the basis of a single trial in which surrogate end points were assessed three hours after administration. In cardiovascular medicine, we learned long ago that therapies directed at surrogate end points — such as the suppression of premature ventricular contractions or, for inotropic agents, an improved ejection fraction — can be associated with excess deaths. With the low threshold set for regulatory approval, the FDA did not demand appropriate warnings on the label regarding an increased risk of death or worsened renal function and did not require the performance of trials that would have provided definitive verification of the safety and efficacy of nesiritide.

We practice medicine in an era in which there is one pharmaceutical-company representative for every five physicians and in which companies will stretch the limits in their marketing of drugs. The boundary lines that previously separated industry from the FDA and academia have unfortunately become blurred. Interestingly, the European Agency for the Evaluation of Medicinal Products, the counterpart of the FDA, has still

not approved nesiritide and awaits the results of a trial involving 1900 patients before it will even consider doing so.[5]

In my view, nesiritide has not yet met the minimal criteria for safety and efficacy. Until a trial definitively proves that this drug reduces the risk of death or repeated hospitalization for heart failure, there will be questions about the appropriateness of the drug's use or even commercial availability. We need a tune-up of our procedures to eliminate indiscriminate use of drugs, such as nesiritide, when there is not proper evidence of their safety.

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An interview with Dr. Topol can be heard at www.nejm.org.

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Financial Conflicts of Interest and the Food and Drug Administration's Advisory Committees

Robert Steinbrook, M.D.

Advisory committees to the Food and Drug Administration (FDA) help the agency make decisions about the approval of medications and medical devices, among other issues. Membership on these committees is subject to detailed policies and procedures for managing potential conflicts of interest and for balancing possible conflicts against the agency's need for advisers with relevant scientific expertise (see box).[1,2] Two recent high-profile meetings have raised questions about the agency's approach and whether it should change.

In February, the FDA convened a joint meeting of the Arthritis Advisory Committee and the Drug Safety and Risk Management Advisory Committee to discuss the safety of cyclooxygenase-2 (COX-2) inhibitors. At the beginning of the meeting, an agency official read a conflict-of-interest statement indicating that in the FDA's judgment the topics were "issues of broad applicability and there [were] no products being approved." Although the FDA acknowledged the possibility of conflicts of interest on the part of committee members, it declared that "because of the general nature of the discussions before the committee, these potential conflicts are mitigated." The agency issued general waivers to the members who required them in order to participate; no specific information was provided.

After the meeting, it was disclosed that 10 of the 32 voting panel members had financial associations with the manufacturers of COX-2 inhibitors, such as the receipt of speaking or consulting fees or research support.[3,4] Of the 30 votes cast by these 10 members on whether rofecoxib, celecoxib, and valdecoxib should continue to be marketed, 28 favored marketing the drugs. Of the 66 votes of the other 22 members, only 37 favored marketing the drugs. If the 10 panel members with the financial associations had not participated, the committee would have voted 12 to 8 that valdecoxib should be withdrawn and 14 to 8 that rofecoxib should not return to the market. With their votes included, the tally was 17 to 13 for keeping valdecoxib on the market and 17 to 15 for the return of rofecoxib.[4] Subsequently, the FDA announced that it had asked Pfizer, the manufacturer of valdecoxib, to withdraw it voluntarily from the market. Rofecoxib, which Merck voluntarily withdrew from the market in 2004, remains off the market.

According to Dr. Alastair J.J. Wood of Vanderbilt University Medical School, the chair of the joint meeting, the FDA made a "judgment error" when it decided to issue a general waiver and not to disclose specific information

Managing Conflicts of Interest for FDA Advisory Panels

The FDA's management of conflicts of interest for its advisory committees is based on the Ethics Reform Act of 1989 and implementing regulations that were issued in 1996 by the Office of Government Ethics.[1,2] Voting members of FDA advisory committees are considered "special government employees." Before each meeting in which they may participate, these experts complete a detailed confidential financial disclosure report. The agency determines whether any of the reported relationships pose a potential conflict of interest, and some people are disqualified on this basis.

The FDA, like other federal agencies, is permitted to balance its needs for scientific expertise against the potential for a conflict and to grant a waiver when "the need for the individual's services outweighs the potential for a conflict of interest."[1] If the FDA determines that only general topics are being discussed, as at the meeting on cyclooxygenase-2 inhibitors, it takes a different approach from that used when it determines that approval of a specific product is being considered.

In reaching decisions, FDA officials use a detailed "waiver criteria document" that provides guidance and suggests an "expected action," which is defined as "the outcome that is anticipated in the majority of cases."[1] For example, when an advisory committee is considering approval of a specific product, stock holdings of greater than $100,000 are expected to lead to exclusion, whereas smaller stock holdings lead to a decision by the agency. A limited waiver permits partial participation. The FDA may allow a member to participate in discussions and deliberations but not to vote.

After granting a waiver, the FDA balances the public's right to the information against the privacy of its advisory committee members. According to the agency, "information to be disclosed will adequately enable a reasonable person to understand the nature of the conflict and the degree to which it could be expected to influence the recommendations the [special government employee] will make."[2]

The disclosure is read into the record at the beginning of the meeting. The FDA usually does not provide specifics when it grants a general waiver. When it grants a specific waiver, it usually discloses the type of interest (such as stock, consulting, or contracts and grants) as well as the magnitude, which is expressed in terms of dollar ranges rather than as a specific amount. The disclosure notes whether the financial interest is related to the product under discussion or a competing product (without naming the competitor). The actual waiver statements are not released; they can be obtained only through a written request under the Freedom of Information Act.

about the potential conflicts of members of the committees. In an interview, Wood said: "Of all the FDA advisory committee meetings I have attended, there has never been more money on the table. Some potential panel members had already been excluded because of conflicts. The people who were chosen had disclosed their financial interests to the FDA, although it played out as though they had something to hide."

Concern about potential conflicts of interest arose again in April, when the General and Plastic Surgery Devices Panel of the Medical Devices Advisory Committee met to consider the safety of silicone gel–filled breast implants made by Inamed and Mentor, both of Santa Barbara, California. Before the meeting, the FDA told a plastic surgeon from George Washington University School of Medicine and Health Sciences that the $50,000 to $100,000 in stock he owned in a company that is seeking to purchase Inamed did not disqualify him, and he was designated as one of 10 voting members of the panel. Days later, the agency said that he could participate but not vote; he declined a nonvoting seat. The remaining plastic surgeon on the panel had a major role in the development of an educational CD-ROM about breast-reconstruction surgery, a project that had received funding from Inamed. At the beginning of the meeting, an FDA official said that the surgeon "reported his institution's past and current involvement with firms at issue. In the absence of personal financial interests, the Agency has determined that he may participate fully in the Panel's deliberations." In the end, the panel made a split recommendation — approval of the implants made by Mentor and nonapproval of those made by Inamed.

In these recent cases, the FDA followed its standard procedures for managing conflicts of interest. The panel members with financial interests said that their ties did not influence their votes.

Nonetheless, the cases raise the specific concern that the agency's disclosure statements are opaque and lack detail. They also raise the general concern that waivers for potential conflicts are common and that the agency has paid insufficient attention to its — and the public's — interest in selecting scientific advisers who are independent of industry.

The FDA has 30 advisory committees and holds nearly 85 advisory committee meetings a year. Voting members of FDA advisory committees are considered "special government employees." In 2003 and 2004, about 12 percent of the special government employees participating in these meetings were granted waivers related to the particular matter before their committee (an average of 194 waivers per year). In May, Dr. Steven Galson, acting director of the FDA's Center for Drug Evaluation and Research, said that the frequent waiver of these conflict-of-interest rules for advisory committees was "very controversial."

The matter is complicated by the importance of the FDA's decisions for medical practice and public health, the agency's need for specialized expertise on specific topics, the huge amounts of money that are often at stake, and the extensive financial ties between leading medical researchers and industry. It is also complicated by the fact that the FDA has been without a permanent commissioner since March 2004. The FDA itself receives substantial financial support from the "user fees" that pharmaceutical companies pay the agency under the Prescription Drug User Fee Act of 1992 and that are used primarily to accelerate drug approvals.[5] As the *New York Times* noted in an editorial on March 4, 2005, "Unless the FDA makes a more aggressive effort to find unbiased experts or medical researchers start severing their ties with industry, a whiff of bias may taint the verdicts of many advisory panels."

Some changes to the FDA's approach to financial conflicts of interest could probably be implemented by the agency or the Department of Health and Human Services. One possible approach would be for the FDA to publish the names and background information of proposed committee members in the *Federal Register* and on its Web site and to give the public several weeks to comment. The agency could then consider these comments before the roster of participants in an advisory meeting was made final. Such procedures for public comment are used by the National Academies and the Environmental Protection Agency. The FDA could also make public more complete financial disclosures for its outside advisers. A possible criticism of such a move is that potential advisers would be less willing to serve under these conditions. However, in recent years, detailed public disclosures have become widely accepted — for example, in articles in medical journals and in materials associated with continuing medical education activities.

Any fundamental change in the FDA's approach, such as excluding from advisory committees anyone who is a paid consultant to industry, would probably require new federal legislation. On June 8, 2005, the House of Representatives, by a vote of 218 to 210, attached a rider to the bill that includes appropriations for the FDA (H.R.2744). The amendment (H.AMDT.235), sponsored by Representative Maurice Hinchey (D-N.Y.), would prohibit the agency from using appropriated funds to grant waivers of its financial conflict-of-interest requirements to voting members of its advisory committees. As of early July, the measure was under consideration in the Senate, where Senator Richard Durbin (D-Ill.) is backing a similar amendment. If the amendment eventually becomes law, industry-connected scientists would be unable to serve on advisory committees during fiscal year 2006 (October 1, 2005, to September 30, 2006), the period covered by the appropriations bill.

Dr. Steinbrook is a national correspondent for the *Journal*.

1. Food and Drug Administration. FDA guidance on conflict of interest for advisory committee members, consultants and experts. February 2000. (Accessed June 23, 2005, at http://www.fda.gov/oc/advisory/conflictofinterest/guidance.html.)
2. *Idem.* Draft guidance on disclosure of conflicts of interest for special government employees participating in FDA product specific advisory committees. January 2002. (Accessed June 23, 2005, at http://www.fda.gov/oc/guidance/advisorycommittee.html.)
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Last-Ditch Medical Therapy — Revisiting Lobotomy

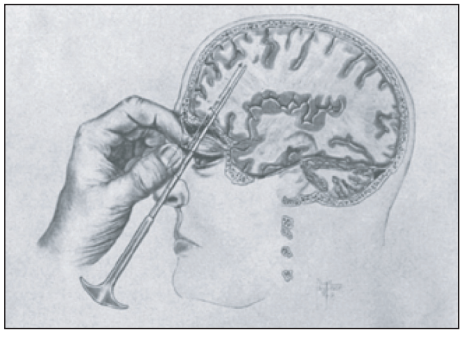
Barron H. Lerner, M.D., Ph.D.

Desperate times call for desperate measures. So thought Walter J. Freeman, a neurologist who became the United States's staunchest advocate of the lobotomy between the 1930s and the 1970s. A new book, *The Lobotomist*, by journalist Jack El-Hai,[1] chronicles Freeman's advocacy of a procedure that was viewed by many, and continues to be viewed, as barbaric. In exploring the ways in which lobotomy became part of common medical practice, El-Hai raises questions not only about how we should judge the procedure in retrospect, but also about what lobotomy teaches us about last-ditch medical interventions.

In the early 1900s, relatives frequently committed their loved ones to long stays in understaffed, overcrowded, and often filthy mental institutions. The therapeutic options for severe mental illness were quite limited.

One option, the lobotomy, also known as leucotomy, was devised in 1935 by the Portuguese neurologist Egas Moniz. It involved drilling holes in the skull and using a blade to sever nerve fibers running from the frontal lobes to the rest of the brain. Moniz believed that psychiatric symptoms were caused by faulty nerve connections established over a period of years. If these nerves were severed and new connections were allowed to form, he postulated, patients' symptoms would improve. Lobotomies were originally used to treat patients with depression but were later often performed to treat schizophrenic patients suffering from agitation and paranoid delusions.

The principal U.S. proponent of lobotomy was Freeman, of George Washington University Medical School. In June 1937, at the annual meeting of the American Medical Association, Freeman and his colleague James W. Watts, a neurosurgeon, presented data on 20 patients who had undergone lobotomy.[2] Their paper launched a fierce debate on the procedure. On the one hand, certain members of the medical profession consistently condemned it as brutal, unscientific, and harmful. This appears to have been the case with the 1941 lobotomy performed on Rosemary Kennedy, the mildly retarded sister of John F. Kennedy, whose cognitive functions were severely worsened by the operation. The negative image of the lobotomy entered the popular culture through Ken Kesey's 1962 novel *One Flew Over the Cuckoo's Nest* and the movie based on it, in which the rebellious hero becomes nearly catatonic after undergoing the operation.



On the other hand, Freeman's data painted quite a different picture. The condition of 13 of the 20 patients, he and Watts claimed, had improved. In one case, a 63-year-old housewife who had had increasing anxiety and agitation for a year, they said, "now manages home and household accounts, enjoys people, attends theater, drives her own car."[2]

Bolstered by such results, which were confirmed by later studies, Freeman's enthusiasm for lobotomy increased. In 1946, he devised the so-called transorbital lobotomy, in which he used a mallet to pound an ice pick through the patient's eye socket into the brain, then moved the pick around blindly to sever the nerve fibers. He traveled the world promoting his new procedure.

Certain physicians, especially those who treated the roughly 400,000 patients in state mental hospitals, embraced the lobotomy. So did the media, thanks in part to Freeman's showmanship. Tens of thousands of lobotomies were performed in the United States before the introduction of chlorpromazine and other neuroleptic medications made the operation all but obsolete by the 1960s. In 1949, Moniz was awarded the Nobel Prize in Physiology or Medicine for inventing the procedure.

One of the virtues of historical scholarship is its dynamism:

each scholar, building on new information and insights, can revise the conclusions of earlier works. The first book to evaluate lobotomy, Elliot S. Valenstein's *Great and Desperate Cures*,[3] was highly critical of Freeman and his operation, which Valenstein saw as providing a cautionary tale about overzealous physicians. Joel Braslow's *Mental Ills and Bodily Cures* argued that a major motivation for lobotomies was to create "apathetic, indifferent, and docile" patients who would be more compliant than they had been.[4] But Jack D. Pressman, in *Last Resort*, emphasized the importance of evaluating historical events within the context of their own time.[5] Although the notion of cutting brain tissue in order to make people submissive is repugnant from our modern perspective, the ability to discharge psychiatric patients even to a limited existence at home was perceived as a therapeutic triumph in the 1940s and 1950s.

Having immersed himself in Freeman's papers, El-Hai found himself, much to his surprise, siding with Pressman. The physician who had been compared to the Nazi doctor Josef Mengele actually appeared to have helped many people. For example, Harry Dannecker, an Indiana man with a long history of anxiety and depression, had been suicidal before he underwent a lobotomy in 1937; during World War II, completely recovered, he worked long hours in a war-materials plant. Among the pieces of evidence stressed by El-Hai are thousands of letters from grateful patients. Freeman and Watts, one wrote, "saved my mind and set my spirit free."

So, was lobotomy a reasonable intervention for a desperate problem or a routine cause of harm, as Christine Johnson, whose grandmother had a lobotomy in 1954, charges? Johnson has founded a Web site, www.psychosurgery.org, that is sponsoring a petition to get Moniz's Nobel Prize revoked.

One difficulty in assessing the procedure arises from the nature of Freeman's research. He kept in touch with as many patients as possible, even traveling across the country to find them. Yet since he conducted no controlled studies, interpreting his data is difficult. For example, since mental illness in any particular patient may wax and wane, it is possible that some patients' symptoms might have improved even if portions of their brains had not been cut away. And grateful letters may represent a skewed sample. Still, it is hard to deny that some patients who had been institutionalized for years lived apparently satisfactory lives after undergoing lobotomy — even, in rare cases, becoming lawyers or physicians, according to El-Hai.

Surely the most disturbing aspect of Freeman's story was his decision to perform lobotomies on unwilling patients. Some of the stories El-Hai recounts are positively gruesome. In 1950, for example, Freeman did a transorbital procedure in a motel room while police held the agitated patient down. As late as the 1960s, he performed lobotomies in otherwise healthy adolescent boys who had been diagnosed with anxiety — an act that surely violated medicine's admonition to "do no harm." To the extent that Freeman's fellow physicians knew about and tolerated such activities, this episode represents a blot on the history of the medical profession.

But whereas Freeman's later excesses raise obvious red flags, his earlier efforts on behalf of a population of very ill patients pose a more complicated question. To what degree should physicians and researchers "push the envelope" in search of an effective remedy? Here the history of lobotomy offers a somewhat surprising answer. Lobotomy was not, as it was long considered, an aberrant and cruel therapy promulgated by fringe practitioners. Rather, it exemplified a common characteristic of medical practice, in which doctors and patients have often felt the need to "do something" in the face of seemingly hopeless situations. In such cases, some patients have inevitably served as guinea pigs. Radical cancer surgery, artificial-heart implantation, and the early organ transplantations come to mind. Sometimes, the interventions are the first step toward a successful remedy; in other instances, they prove worthless.

In this sense, Freeman's story is less a cautionary tale of a doctor gone wrong than a cautionary tale of business as usual in medicine. Last-ditch medical interventions will probably always be with us. We must therefore continue to scrutinize them, not only in ret-

rospect but as they are being conceptualized, publicized, and carried out.

Dr. Lerner is an associate professor of medicine and public health at the Columbia University Medical Center, New York.

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THIS WEEK in the JOURNAL

ORIGINAL ARTICLE

Erlotinib in Previously Treated Non–Small-Cell Lung Cancer

The efficacy of erlotinib in patients with non–small-cell lung cancer and relapse after treatment with conventional chemotherapy was tested in a placebo-controlled, double-blind trial. Erlotinib, an inhibitor of the epidermal growth factor receptor, was associated with responses in about 9 percent of patients and with prolonged survival in some cases.

The futility of further treatment of patients with non–small-cell lung cancer after a failure of response to one or two different chemotherapy regimens is well documented. This trial shows that erlotinib can help such patients and suggests further studies of the drug in less advanced disease.

SEE P. 123; EDITORIAL, P. 200

ORIGINAL ARTICLE

Erlotinib in Lung Cancer — Molecular and Clinical Predictors of Outcome

This companion to the clinical trial of erlotinib in patients with non–small-cell lung cancer studied the epidermal growth factor receptor (EGFR) protein and gene (*EGFR*) in tumor specimens obtained from the participants. Expression of EGFR by the tumor was associated with responsiveness to the drug but not with increased survival. Neither the number of copies of *EGFR* nor mutational status was associated with responsiveness or survival.

The striking finding in this study is that, in contrast to previous trials involving small numbers of patients, there was no association between the presence of *EGFR* mutations and the likelihood of a response to an EGFR inhibitor.

SEE P. 133; EDITORIAL, P. 200

ORIGINAL ARTICLE

Single-Chamber versus Dual-Chamber Pacing for High-Grade Atrioventricular Block

Patients with high-grade atrioventricular block usually require the implantation of a permanent pacemaker. Retrospective studies have suggested that dual-chamber pacemakers reduce the risk of atrial fibrillation, stroke, heart failure, and death in this setting, as compared with single-chamber ventricular pacemakers. In a randomized trial comparing these two pacing methods, however, no significant advantage of dual-chamber pacing was demonstrated.

SEE P. 145; EDITORIAL, P. 202; CME, P. 218

BRIEF REPORT

Relapsing Systemic Inflammation and Human Herpesvirus 8 Infection

A 61-year-old immunocompetent woman had recurrent episodes of fever, lymphadenopathy, splenomegaly, synovitis, and rash. Although she was negative for human immunodeficiency virus infection, Kaposi's sarcoma developed. The relapsing inflammatory symptoms were associated with sharp increases in the levels of human herpesvirus 8 in plasma and of peripheral-blood mononuclear cells.

SEE P. 156

CLINICAL PRACTICE

Screening for Osteoporosis

At her annual visit, a 60-year-old woman asks her physician whether she should have a bone-density test to screen for osteoporosis. The patient went through menopause at the age of 52 years and received postmenopausal hormone therapy for four years. She takes 500 mg of calcium twice daily and exercises regularly. She has no personal history of fractures, but her mother had a hip fracture at the age of 82. Her height is 63 in. and her weight is 120 lb. What should her physician advise?

SEE P. 164; CME, P. 217

MECHANISMS OF DISEASE

Tyrosine Kinases as Targets for Cancer Therapy

Tyrosine kinases, enzymes that catalyze the transfer of phosphate from ATP to tyrosine residues in polypeptides, are ubiquitous, numerous, and of considerable clinical interest because they participate in the development of cancer and have become choice targets for therapeutic intervention. This comprehensive review discusses the molecular and clinical aspects of tyrosine kinases.

SEE P. 172; CME, P. 219

CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL

A Male Infant with Jaundice and Thrombocytopenia

A four-week-old male infant was admitted to the hospital because of jaundice and abdominal distention. Magnetic resonance imaging revealed decreased signal in the liver, pancreas, and heart, with nodularity of the liver. A diagnostic procedure was performed.

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Erlotinib in Previously Treated Non–Small-Cell Lung Cancer

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ABSTRACT

BACKGROUND

We conducted a randomized, placebo-controlled, double-blind trial to determine whether the epidermal growth factor receptor inhibitor erlotinib prolongs survival in non–small-cell lung cancer after the failure of first-line or second-line chemotherapy.

METHODS

Patients with stage IIIB or IV non–small-cell lung cancer, with performance status from 0 to 3, were eligible if they had received one or two prior chemotherapy regimens. The patients were stratified according to center, performance status, response to prior chemotherapy, number of prior regimens, and prior platinum-based therapy and were randomly assigned in a 2:1 ratio to receive oral erlotinib, at a dose of 150 mg daily, or placebo.

RESULTS

The median age of the 731 patients who underwent randomization was 61.4 years; 49 percent had received two prior chemotherapy regimens, and 93 percent had received platinum-based chemotherapy. The response rate was 8.9 percent in the erlotinib group and less than 1 percent in the placebo group (P<0.001); the median duration of the response was 7.9 months and 3.7 months, respectively. Progression-free survival was 2.2 months and 1.8 months, respectively (hazard ratio, 0.61, adjusted for stratification categories; P<0.001). Overall survival was 6.7 months and 4.7 months, respectively (hazard ratio, 0.70; P<0.001), in favor of erlotinib. Five percent of patients discontinued erlotinib because of toxic effects.

CONCLUSIONS

Erlotinib can prolong survival in patients with non–small-cell lung cancer after first-line or second-line chemotherapy.

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*The investigators and centers participating in this National Cancer Institute of Canada Clinical Trials Group study are listed in the Appendix.

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LUNG CANCER IS THE LEADING CAUSE OF cancer death among men and women in North America.[1] In advanced non–small-cell lung cancer, chemotherapy offers symptomatic relief and modest improvement in survival[2]; responses are brief, with a median time to progression of three to five months. Second-line chemotherapy with docetaxel can prolong survival after platinum-based therapy for non–small-cell lung cancer.[3,4] However, there is at present no defined role for third-line chemotherapy. The futility of offering third-line chemotherapy was demonstrated by Massarelli et al.,[5] who reported a response rate of only 2 percent and a median survival of four months. Shepherd et al.[6] showed that among patients treated with docetaxel after the failure of two or more chemotherapy regimens, survival was identical to that among patients treated with supportive care.

The epidermal growth factor receptor (EGFR) family is part of a complex signal-transduction network that is central to several critical cellular processes. Since EGFR is often found in non–small-cell lung cancer cells,[7,8] it has been the focus of efforts to develop new agents that target the EGFR pathway. Erlotinib (Tarceva, OSI Pharmaceuticals) and gefitinib (Iressa, AstraZeneca) inhibit the tyrosine kinase activity of EGFR and have been studied extensively.[9-12] In randomized phase 2 trials of gefitinib (Iressa Dose Evaluation in Advanced Lung Cancer [IDEAL] 1 and 2),[10,11] the tumors of 10 to 20 percent of patients who were previously treated with platinum-based regimens responded, and in a phase 2 trial of erlotinib among previously treated patients with non–small-cell lung cancer in which 10 percent or more of the cells expressed EGFR, the response rate was 12.3 percent.[12] These promising rates are perhaps higher than those possible with other forms of chemotherapy,[3-6] but it is unknown whether treatment with an EGFR inhibitor prolongs survival. For this reason, the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) conducted a trial (BR.21) to compare erlotinib with placebo after the failure of standard chemotherapy for non–small-cell lung cancer. The inclusion of a control group receiving placebo was considered ethical in view of the lack of benefit from further chemotherapy after the failure of standard treatment.[5,6]

METHODS

STUDY DESIGN

This international, phase 3, randomized, double-blind, placebo-controlled trial of erlotinib after the failure of first-line or second-line chemotherapy for non–small-cell lung cancer was designed by the NCIC CTG. Patients were randomly assigned in a 2:1 ratio to receive oral erlotinib at a dose of 150 mg daily or placebo. Randomization was performed centrally by Applied Logic Associates (Houston), with the use of the minimization method.[13] Patients were stratified according to center, Eastern Cooperative Oncology Group performance status (0 or 1 vs. 2 or 3, with higher scores indicating greater impairment), best response to prior therapy (complete or partial response vs. stable disease vs. progressive disease), number of prior regimens received (one vs. two), and exposure to prior platinum therapy (yes vs. no).

The primary end point was overall survival. Secondary end points included progression-free survival, overall response rate (complete and partial), duration of response, toxic effects, and quality of life. Responses were assessed with the use of the Response Evaluation Criteria in Solid Tumors (RECIST),[14] and toxic effects were assessed according to the Common Toxicity Criteria of the National Cancer Institute (version 2.0). The European Organization for Research and Treatment of Cancer (EORTC) quality-of-life questionnaire (QLQ-C30) and the quality-of-life questionnaire for patients with lung cancer (QLQ-LC13) were used to evaluate patients' quality of life.

The protocol was approved by the ethics review boards at all participating institutions, and all patients provided written informed consent. Support was provided by the NCIC and OSI Pharmaceuticals. Data were collected, managed, and analyzed by the NCIC CTG, and the manuscript was written by members of the NCIC CTG. OSI Pharmaceuticals reviewed the final manuscript and provided comments on it. Confidentiality was maintained by both the NCIC CTG and OSI Pharmaceuticals. The study chair, Dr. Shepherd, and the physician coordinator, Dr. Seymour, reviewed all the data and confirmed their completeness and accuracy.

ELIGIBILITY CRITERIA

Patients 18 years of age or older with an Eastern Cooperative Oncology Group (ECOG) performance status between 0 and 3 were eligible in the presence of documented pathological evidence of non–small-cell lung cancer. The patients had to have received one or two regimens of combination chemotherapy and not be eligible for further chemotherapy. Patients 70 years of age or older may have received therapy with one or two single agents. Patients had to have recovered from any toxic effects of therapy and were randomly assigned to the study treatment at least 21 days after chemotherapy (14 days after treatment with vinca alkaloids or gemcitabine) and 7 days after radiation. Adequate hematologic and biochemical values were required.

Patients with prior breast cancer, melanoma, or hypernephroma were ineligible, as were those with other malignant diseases (except basal-cell skin cancers) within the preceding five years. Other exclusion criteria were symptomatic brain metastases, clinically significant cardiac disease within one year, ventricular arrhythmias requiring medication, and clinically significant ophthalmologic or gastrointestinal abnormalities.

STUDY PROCEDURES

Within seven days before randomization, a history and physical examination were obtained and hematologic and biochemical testing, chest radiography, and assessments of toxic effects and quality of life were obtained. Computed tomographic scans of the chest and abdomen were obtained within 28 days before randomization. For a patient to be evaluated for a response, at least one measurable lesion was required, but measurable disease was not mandatory for eligibility. Only patients with measurable disease were included in the analyses of complete or partial response.

Administration of the study medication was to start within two days after randomization. For grade 2 diarrhea, loperamide was recommended without reduction of the dose of erlotinib. For grade 3 diarrhea, the study treatment was withheld until the diarrhea was grade 1 or less, and then erlotinib at a dose of 100 mg daily was started. For grade 1 or 2 rash, treatment modification was not recommended. For grade 3 rash, treatment was withheld, the rash was treated symptomatically, and erlotinib at a dose of 100 mg daily was restarted when the rash was grade 1 or less.

History taking, physical examination, and hematologic and biochemical testing were performed every four weeks, and radiologic investigations every eight weeks. Patients' quality of life was evaluated every four weeks in countries with validated versions of the questionnaires.

EGFR EXPRESSION

Separate written consent for optional tissue banking and correlative studies was obtained. EGFR expression was determined with the use of immunohistochemistry in a central laboratory that used Dako kits (DakoCytomation). Positivity was defined as more than 10 percent of cells staining at any intensity for EGFR.

STATISTICAL ANALYSIS

The trial was designed to detect, with 90 percent power and a two-sided type I error of 5 percent, a 33 percent improvement in median survival from four months as estimated in the placebo group. For the final analysis, 582 deaths were required and were projected to occur with a sample size of 700 patients enrolled over a period of 14 months with 6 months of follow-up. The required number of deaths had occurred by January 2004, and the database was locked as of April 23, 2004. There was no interim analysis. Tumor responses were validated centrally for the first 333 patients in the trial.

The stratified log-rank test, accounting for stratification factors at randomization (except center) and EGFR protein expression (positive vs. negative vs. unknown), was used to compare progression-free survival and overall survival between treatment groups. Exploratory forward stepwise regression analyses with the use of the Cox model were performed to adjust for treatment effect and to identify prognostic factors for progression-free survival and overall survival. Candidate covariates included EGFR expression, stratification factors (except center), sex, age (60 years or less vs. more than 60 years), race or ethnic group (Asian vs. others), prior radiotherapy (yes vs. no), histologic subtype of cancer (adenocarcinoma vs. others), and smoking status (smoker vs. nonsmoker vs. unknown). Race was self-reported or determined by study personnel and was not based on country of domicile. Fisher's exact test was used to compare response rates between levels of potential predictors and rates of toxic effects between treatments. Times to deterioration (a 10-point increase from the baseline score) for

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Erlotinib (N=488)	Placebo (N=243)
Age (yr)		
Median	62	59
Range	34–87	32–89
<60 (% of patients)	42.6	51.0
≥60 (% of patients)	57.4	49.0
Sex (% of patients)		
Male	64.5	65.8
Female	35.5	34.2
Race or ethnic group (% of patients)†		
Asian	12.9	12.2
Other	87.1	87.8
Performance status (% of patients)‡		
0	13.1	14.0
1	52.5	54.3
2	25.8	23.0
3	8.6	8.6
Weight loss >10% (% of patients)	11.0	12.0
Pathological subtype (% of patients)		
Adenocarcinoma	50.4	49.0
Squamous-cell carcinoma	29.5	32.1
Other	20.1	18.9
Prior chemotherapy (% of patients)		
One regimen	50.6	50.2
Two or more regimens	49.4	49.8
Platinum-based therapy	92.0	91.8
Response to prior chemotherapy (% of patients)		
Complete or partial response	38.1	37.9
Stable disease	34.0	34.2
Progressive disease	27.9	28.0
Smoking status (% of patients)		
Current smoker or ever smoked	73.4	77.0
Never smoked	21.3	17.3
Unknown	5.3	5.8
EGFR protein expression (% of patients)§		
Positive	24.0	27.6
Negative	19.1	19.8
Unknown	56.9	52.6

* Because of rounding, not all percentages sum to 100.
† Race or ethnic group was self-reported or determined by study personnel and was not based on country of domicile.
‡ A higher score indicates greater impairment.
§ Epidermal growth factor receptor (EGFR) expression was assessed by immunohistochemistry.

cough, dyspnea, and pain were identified prospectively as the primary end points for the analysis of quality of life[15] and were analyzed with the use of the log-rank test, with adjustment according to the Hochberg method[16] for the comparison of multiple end points. All P values were two-sided.

RESULTS

PATIENT CHARACTERISTICS

Between August 2001 and January 2003, 731 patients were randomly assigned to erlotinib (488) or placebo (243). Twenty-two patients (12 assigned to erlotinib and 10 assigned to placebo) were ineligible for the following reasons: three prior chemotherapy regimens (9); single-agent chemotherapy for patients less than 70 years of age (2); inadequate time since the last treatment (5); abnormal biochemistry results (4); and symptomatic brain metastases (2). All 731 patients were included in the efficacy analyses, and 727 treated patients (485 assigned to erlotinib and 242 assigned to placebo) were included in the safety analyses. Eight patients assigned to erlotinib (1.6 percent) and 18 assigned to placebo (7.4 percent) received other EGFR inhibitors after study medication was discontinued. The groups were balanced with respect to baseline characteristics and important prognostic variables (Table 1).

RESPONSE AND SURVIVAL

In patients with at least one target lesion, the lesions were evaluated according to RECIST (427 patients assigned to erlotinib and 211 assigned to placebo). In the erlotinib group, the rates of complete response and partial response were 0.7 percent and 8.2 percent, respectively (median duration, 7.9 months); in the placebo group, the rate of partial response was less than 1 percent (P<0.001), but these responses were not externally validated. In an intention-to-treat analysis of all patients randomly assigned to treatment, the disease-control rate (i.e., the rate of complete or partial responses and stable disease) in the erlotinib group was 45 percent; 38 percent of the patients had progressive disease, and among the remaining 17 percent progression was not confirmed. The likelihood of a response to erlotinib (Table 2) among patients with non–small-cell lung cancer was not significantly altered by performance status, prior treatments, prior response, or age, but it was higher among women (P=0.006), nonsmokers (P<0.001), Asians

(P=0.02), patients with adenocarcinoma (P<0.001), and patients in whom 10 percent or more of the tumor cells expressed EGFR (P=0.10). In multiple logistic-regression analyses, never having smoked (P<0.001), the presence of adenocarcinoma (P=0.01), and EGFR expression (P=0.03) were associated with responsiveness to erlotinib.

At the time of analysis, 587 deaths had occurred (378 in the erlotinib group and 209 in the placebo group). Figure 1 shows Kaplan–Meier curves for overall survival and progression-free survival. Median overall survival in the erlotinib group was 6.7 months, and in the placebo group it was 4.7 months (adjusted hazard ratio, 0.70; 95 percent confidence interval, 0.58 to 0.85; P<0.001). In the Cox regression analysis, erlotinib remained associated with longer survival (P=0.002), as did Asian origin (P=0.01), adenocarcinoma on histologic examination (P=0.004), and never having smoked (P=0.048 vs. current or past smoking). Table 3 shows the exploratory subgroup analyses. Although the sample sizes may be inadequate to detect small or moderate differences, a benefit from erlotinib was apparent in most of the subgroups. The interaction between treatment and the covariate defining the subgroup was statistically significant only for smoking status. At the time of analysis, 682 patients had had progression of disease (450 in the erlotinib group and 232 in the placebo group). Median progression-free survival was 2.2 months in the erlotinib group and 1.8 months in the placebo group (adjusted hazard ratio, 0.61; 95 percent confidence interval, 0.51 to 0.74; P<0.001). In the Cox model, treatment with erlotinib (P<0.001) and never having smoked (P<0.01 for the comparison with current or past smoking) were associated with longer progression-free survival.

TOXIC EFFECTS

Four patients who underwent randomization did not receive treatment. Table 4 shows that 19 percent of the erlotinib group required dose reductions because of drug-related toxic effects, as compared with 2 percent of the placebo group, most frequently because of rash (12 percent) and diarrhea (5 percent); 26 patients (5 percent) discontinued erlotinib because of drug-related toxic effects, as compared with 4 patients (2 percent) receiving placebo. There was a higher incidence of infection among the erlotinib patients, which may reflect longer follow-up (P<0.001). There were similar rates of pneumonitis and pulmonary fibrosis in the two groups.

Table 2. Analysis of Responses to the Study Treatment.*

Factor	No. of Cases Evaluated	No. of Responses (Complete and Partial)	Overall Response Rate (%)	P Value
Treatment				
Erlotinib	427	38	8.9	<0.001
Placebo	211	2	<1	
Age				
<60 yr	177	19	10.7	0.30
≥60 yr	250	19	7.6	
Sex				
Male	281	17	6.0	0.006
Female	146	21	14.4	
Pathological subtype				
Adenocarcinoma	209	29	13.9	<0.001
Other	218	9	4.1	
Performance status				
0 or 1	274	21	7.7	0.29
2 or 3	153	17	11.1	
Response to prior therapy				
Complete and partial responses	174	13	7.5	0.65
Progressive disease	87	9	10.3	
Stable disease	166	16	9.6	
Prior regimens				
1	214	19	8.9	1.00
2 or 3	213	19	8.9	
Prior platinum-based therapy				
Yes	396	36	9.1	1.00
No	31	2	6.5	
EGFR expression†				
Positive	106	12	11.3	0.10
Negative	80	3	3.8	
Unknown	241	23	9.5	
Smoking status				
Current smoker or ever smoked	311	12	3.9	<0.001
Never smoked	93	23	24.7	
Unknown	23	3	13.0	
Race or ethnic group				
Asian	53	10	18.9	0.02
Other	374	28	7.5	

* Responses were assessed according to the Response Evaluation Criteria in Solid Tumors in patients with one or more confirmed lesions and at least one follow-up radiologic examination.

† Epidermal growth factor receptor (EGFR) expression was assessed by immunohistochemistry.

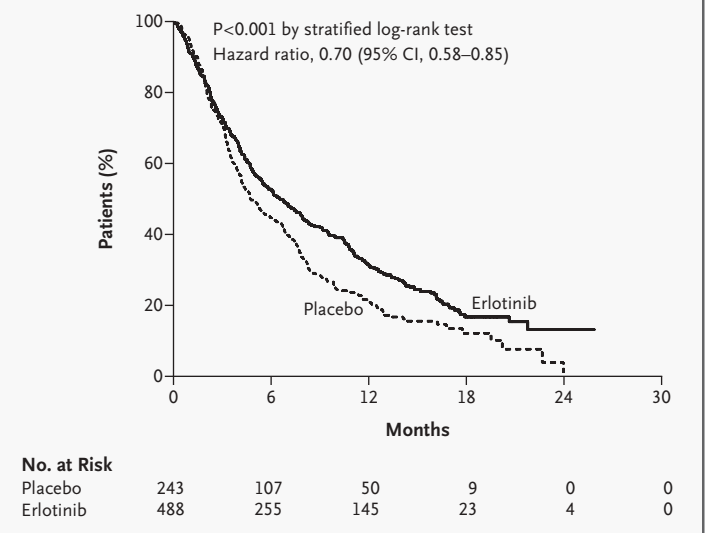

Figure 1. Kaplan–Meier Curves for Overall Survival (Panel A) and Progression-free Survival (Panel B) among All Patients Randomly Assigned to Erlotinib or Placebo.

P values were adjusted for stratification factors (except center) and epidermal growth factor receptor expression.

Two patients died of pneumonitis (one in each group).

QUALITY OF LIFE

Compliance was similar in the two groups. Patients who had responded to the quality-of-life questionnaire at baseline and had one follow-up assessment were included in the analysis. The median time to deterioration with regard to cough (4.9 months among patients receiving erlotinib and 3.7 months among those receiving placebo, P=0.04 with Hochberg adjustment), dyspnea (4.7 months and 2.9 months, respectively; adjusted P=0.03), and pain (2.8 months and 1.9 months, respectively; adjusted P=0.04) in favor of erlotinib. These results are consistent with response-based analyses of the quality of life, which found that more patients receiving erlotinib had improvement in cough, pain, and dyspnea and in the domain of overall physical function (further information is in the Supplementary Appendix, available with the complete text of this article at www.nejm.org).

DISCUSSION

Docetaxel is the only agent known to prolong survival among patients with disease progression after cisplatin-based chemotherapy for non–small-cell lung cancer.[3,4,17] Few options are available for the treatment of patients with disease progression after docetaxel or those who are not eligible for second-line chemotherapy.[5,6] Clearly, new treatments are needed for such patients.

Expression of EGFR is common in non–small-cell lung cancer.[18-20] Several agents that target EGFR are in various phases of clinical evaluation.[9,21] The orally active EGFR tyrosine kinase inhibitors gefitinib and erlotinib have been evaluated in several trials. In the IDEAL 1 trial,[10] patients with non–small-cell lung cancer with disease progression after platinum-based chemotherapy were randomly assigned to receive gefitinib, at a dose of 250 mg or 500 mg daily. There were no differences between the two doses with respect to response rate, time to progression, or median survival. The response rates were also similar whether gefitinib was used as second-line treatment (17.9 percent of patients) or third-line treatment (19.8 percent of patients). In the IDEAL 2 trial,[11] which enrolled symptomatic patients in whom two or more chemotherapy regimens containing platinum and docetaxel had failed, the response rates were 12 percent and 9 percent, respectively, for the two dose levels. More adverse events were seen with the dose of 500 mg in both trials, but discontinuation of treatment because of toxic effects was uncommon at either dose. In a phase 2 trial of erlotinib, the response rate was 12 percent, and response did not correlate with level of EGFR in the tumor.[12]

In our trial, the response rate of 8.9 percent was similar to rates reported for erlotinib and ge-

Table 3. Analysis of Survival.*

Factor	No. of Patients	Univariate Hazard Ratio (95% CI)†	P Value	Multivariate Hazard Ratio (CI)‡	P Value§
Treatment group					
Erlotinib	488	0.7 (0.6–0.9)	<0.001	0.7 (0.6–0.9)	0.002
Placebo	243				
Age				NI	
<60 yr	332	0.8 (0.6–1.0)	0.04		
≥60 yr	399	0.8 (0.6–1.0)	0.02		
Sex				NI	
Male	475	0.8 (0.6–0.9)	0.01		
Female	256	0.8 (0.6–1.1)	0.13		
Pathological subtype					
Adenocarcinoma	365	0.7 (0.6–0.9)	0.008	0.8 (0.6–0.9)	0.004
Other	366	0.8 (0.6–1.0)	0.07		
Performance status				NA	
0 or 1	486	0.7 (0.6–0.9)	0.003		
2	182	0.8 (0.5–1.1)	0.11		
3	63	0.8 (0.4–1.3)	0.33		
Response to prior therapy				NA	
Complete response or partial response	292	0.7 (0.5–0.9)	0.004		
Stable disease	287	0.8 (0.6–1.1)	0.18		
Progressive disease	152	0.9 (0.6–1.2)	0.34		
Prior regimens				NA	
1	369	0.8 (0.6–1.1)	0.03		
2 or 3	362	0.8 (0.6–1.1)	0.02		
Prior platinum-based therapy				NA	
Yes	672	0.7 (0.6–0.9)	<0.001		
No	59	1.7 (0.7–2.7)	0.30		
EGFR expression				NA	
Positive	184	0.7 (0.5–0.9)	0.02		
Negative	141	0.9 (0.6–1.4)	0.70		
Unknown	406	0.8 (0.6–1.0)	0.03		
Smoking status					
Current smoker or ever smoked	545	0.9 (0.7–1.0)	0.14	Reference group	
Never smoked	146	0.4 (0.3–0.6)	<0.001	0.8 (0.6–1.0)	0.048
Unknown	40	1.1 (0.5–2.6)	0.80	1.0 (0.7–1.5)	0.89
Race or ethnic group					
Asian	91	0.6 (0.4–1.0)	0.06	0.7 (0.5–0.9)	0.01
Other	640	0.8 (0.7–0.9)	0.01		

* CI denotes confidence interval, NI not included in the final model, and NA not applicable as a stratification factor.
† The univariate hazard ratio was derived from a Cox model with a single treatment covariate.
‡ The hazard ratio between levels of respective covariates was derived from the final stratified Cox regression model.
§ P values are for the comparison of patients who had never smoked and those whose history of smoking was unknown with those who were smokers.

Table 4. Toxic Effects and Dose Modifications among 727 Patients Receiving the Study Drugs.

Toxic Effect	Erlotinib (N=485)		Placebo (N=242)		P Value	
	All	Grades 3 to 5	All	Grades 3 to 5	All	Grades 3 to 5
	percent					
Rash	76	9	17	0	<0.001	<0.001
Anorexia	69	9	56	5	<0.001	0.06
Nausea	40	3	34	<1	0.12	0.07
Vomiting	25	3	23	2	0.52	0.45
Stomatitis	19	<1	3	0	<0.001	0.31
Diarrhea	55	6	19	<1	<0.001	<0.001
Dehydration	7	4	6	3	0.64	0.67
Ocular toxic effect	28	1	9	<1	<0.001	0.67
Fatigue	79	19	74	23	0.22	0.33
Infection	34	2	21	5	<0.001	0.03
Pulmonary fibrosis	3	<1	3	0	1.0	1.0
Pneumonitis or pulmonary infiltrates*	3	<1	3	<1	0.64	1.0
Death from pneumonitis		1 patient		1 patient		
Reason for dose reduction						
Any toxic effect		19		2		<0.001
Diarrhea		5		0		<0.001
Rash		12		0		<0.001
Conjunctivitis		1		0		0.19
Vomiting		1		0		0.55
Stomatitis		<1		0		1.0
Reason for treatment interruption						
Any toxic effect		27		5		<0.001
Diarrhea		6		<1		
Rash		14		0		<0.001
Conjunctivitis		1		0		0.19
Vomiting		2		<1		0.11
Stomatitis		<1		<1		1.0
Treatment discontinued because of any toxic effect		5		2		0.02

* All cases designated "pneumonitis" were reviewed by a study physician. Cases of "pneumonia" were also reviewed and reclassified as pneumonitis, if appropriate.

fitinib.[10-12] Some investigators have reported that responsiveness to EGFR inhibitors correlates with sex, histologic type, race or ethnic origin, and smoking status.[10,11,21] We also found that response was higher among Asians, women, patients with adenocarcinoma, and lifetime nonsmokers. Contrary to previous reports,[12] the response rate in our trial was higher when 10 percent or more of tumor cells expressed EGFR.

Activating mutations in the *EGFR* gene have been found to predict a response to gefitinib.[22-27] The results of our assays for the number of copies and mutation status of the *EGFR* gene are published in this issue of the *Journal*.[28] Higher response rates were found among patients with high numbers of gene copies and mutations, but the difference was significant only for gene copies.

Because none of the early trials[10-12] had a placebo control group, it is not possible to determine whether EGFR-inhibitor therapy was superior to

palliative treatment. In our placebo-controlled trial, erlotinib did provide clinically meaningful prolongation of survival. According to the Kaplan–Meier estimates, the median survival was prolonged by two months, and 31 percent of patients treated with erlotinib were alive at one year, as compared with 22 percent in the placebo group. The two-month prolongation of survival is similar to that achieved with docetaxel in the setting of second-line chemotherapy,[3,4] even though half the patients in our trial were treated after both first-line and second-line chemotherapy. In this trial and another trial,[3] a significant prolongation of survival was achieved despite response rates of less than 10 percent, perhaps because a high proportion of the patients had durable stable disease while receiving treatment. Survival in this trial appears to be longer than what was achieved in a similar trial of gefitinib, although the response rates were similar in both studies. The characteristics of the patients in these two trials, however, may have differed somewhat.[29]

Exploratory multivariate analyses showed that only Asian origin, adenocarcinoma on histologic examination, and a history of not smoking were significant independent predictors of survival after adjustment for treatment and other potential predictors. Erlotinib had a beneficial effect on survival in almost all subgroups tested, but only the interaction between smoking status and treatment was significantly predictive of a differential effect on survival. Notably, the presence of *EGFR* gene mutations was not predictive of a survival benefit from erlotinib in our study.[28]

In the IDEAL 2 trial,[11] gefitinib rapidly reduced symptoms in 35 percent to 43 percent of patients. In our trial, significantly more patients in the erlotinib group than in the placebo group had reductions in dyspnea, pain, and cough. Furthermore, the time to exacerbation of these symptoms was significantly longer in the erlotinib group. The analysis of the quality of life showed that symptom improvement was also associated with significantly improved physical function.

Rash[30] and diarrhea are the main toxic effects of EGFR inhibitors.[9] They led to dose reduction in 12 percent and 5 percent of patients, respectively, in our trial. Pneumonitis has been reported mainly in Japan following treatment with gefitinib.[31] However, four trials of gefitinib or erlotinib combined with chemotherapy for non–small-cell lung cancer reported similar rates of pneumonitis in active-treatment and placebo groups.[32-35] We rarely encountered pneumonitis or pulmonary fibrosis.

In summary, this trial shows that erlotinib, an oral tyrosine kinase inhibitor of EGFR, prolongs survival and decreases symptoms, as compared with placebo, in previously treated patients with non–small-cell lung cancer.

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APPENDIX

The following investigators and centers participated in this National Cancer Institute of Canada Clinical Trials Group study: A.M. Alvarez, J.L. Martinez, M. Varela, M. Van Kooten, S. Jovtis, Buenos Aires; M. Freue, Lanus, Argentina; M. Rosenthal, Parkville, Australia; D. Yip, Garran, Australia; R. De Boer, Footscray, Australia; S. Ackland, Waratah, Australia; P. Clingan, Wollongong, Australia; D. Campos, San Isidro, Argentina; A. del Giglio, Santo Andre, Brazil; C. Mathias, Bahia, Brazil; I.L Santoro, J.R. Pereira, São Paulo; A.M. Murad, Belo Horizonte, Brazil; R.D.A. Ribeiro, Ceara, Brazil; S. Lago, Porto Alegre, Brazil; J. Vinholes, Porto Alegre, Brazil; R. Martins, Rio de Janeiro; S. Rorke, St. John's, Canada; W. Morzycki, Halifax, Canada; F. Laberge, R. Whittom, Ste.-Foy, Canada; V. Hirsh, J. Ayoub, Montreal; R.W. Gregg, Kingston, Canada; S. Laurie, Ottawa; F. Wierzbicki, Oshawa, Canada; B. Findlay, St. Catharines, Canada; A. Arnold, Hamilton, Canada; L.A. Zibdawi, Newmarket, Canada; J. Meharchand, F. Shepherd, R.L. Burkes, Y.C. Ung, Toronto; J.J. Wilson, Weston, Canada; B. Pressnail, Barrie, Canada; R. Myers, Mississauga, Canada; J. Noble, Sudbury, Canada; D. Walde, Sault Ste. Marie, Canada; S. Navaratnam, Winnipeg, Canada; H.I. Chalchal, Regina, Canada; D.G. Morris, Calgary, Canada; M. Smylie, Edmonton, Canada; D. Fenton, Kelowna, Canada; H. Martins, Victoria, Canada; J. Gutierrez, Las Condes, Chile; U. Gatzemeier, Grobhansdorf, Germany; J. von Pawel, Gauting, Germany; R. Loddenkemper, Berlin; A. Gerogianni, Athens; R. Chan, Pokfulam, Hong Kong; D. Flex, Petach Tikva, Israel; D. Loven, Afula, Israel; M.C. Wollner, Haifa, Israel; H. Biran, Rehovot, Israel; M. Levitt, Tel Hashomer, Israel; A. Cyjon, Zerifin, Israel; J. Lopez, Dviango, Mexico; J. Robles, Mexico City; G. Calderillo, Tlalpan, Mexico; T. Christmas, Auckland, New Zealand; A.B. Simpson, Wellington, New Zealand; M. Dediu, Bucharest, Romania; T.-E. Ciuleanu, Cluj-Napaca, Romania; M. Patran, Sibiu, Romania; L. Miron, Lasi, Romania; L. Ek, Lund, Sweden; B. Bergman, Gothenburg, Sweden; R. Soo, E.H. Tan, Singapore; S. Maoleekoonpairoj, A. Cheirsilpa, Bangkok, Thailand; S. Thongprasert, Chiangmai, Thailand; C.M. Rudin, Chicago; P. Ruff, Johannesburg, South Africa; D.J. Hacking, Westridge, South Africa; S.J. Fourie, Pretoria, South Africa; C. Jacobs, Port Elizabeth, South Africa; C.F. Slabber, Brooklyn Square, South Africa; D.A. Vorobiof, Parklands, South Africa; K. Chi-Hei, Pokfulam, Hong Kong; W. Arpornwirat, Bangkok, Thailand; M. Zukin, Rio de Janeiro.

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ORIGINAL ARTICLE

Erlotinib in Lung Cancer — Molecular and Clinical Predictors of Outcome

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ABSTRACT

BACKGROUND

A clinical trial that compared erlotinib with a placebo for non–small-cell lung cancer demonstrated a survival benefit for erlotinib. We used tumor-biopsy samples from participants in this trial to investigate whether responsiveness to erlotinib and its impact on survival were associated with expression by the tumor of epidermal growth factor receptor (EGFR) and *EGFR* gene amplification and mutations.

METHODS

EGFR expression was evaluated immunohistochemically in non–small-cell lung cancer specimens from 325 of 731 patients in the trial; 197 samples were analyzed for *EGFR* mutations; and 221 samples were analyzed for the number of *EGFR* genes.

RESULTS

In univariate analyses, survival was longer in the erlotinib group than in the placebo group when EGFR was expressed (hazard ratio for death, 0.68; P=0.02) or there was a high number of copies of *EGFR* (hazard ratio, 0.44; P=0.008). In multivariate analyses, adenocarcinoma (P=0.01), never having smoked (P<0.001), and expression of *EGFR* (P=0.03) were associated with an objective response. In multivariate analysis, survival after treatment with erlotinib was not influenced by the status of EGFR expression, the number of *EGFR* copies, or *EGFR* mutation.

CONCLUSIONS

Among patients with non–small-cell lung cancer who receive erlotinib, the presence of an *EGFR* mutation may increase responsiveness to the agent, but it is not indicative of a survival benefit.

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The epidermal growth factor receptor family of genes (*EGFR*) encodes widely expressed transmembrane molecules that have been implicated in the development and progression of cancer.[1-4] After ligand binding, the transmembrane receptor forms homodimers or heterodimers, internalizes, and autophosphorylates tyrosine residues in its cytoplasmic domain, thereby triggering a cascade that leads to cellular proliferation, angiogenesis, metastasis, and inhibition of apoptosis.[2]

The *EGFR* gene is frequently expressed in solid tumors, and in some tumors, expression of the gene correlates with a poor clinical outcome.[5] Non–small-cell lung cancer frequently expresses *EGFR*,[6-14] and for this reason, it is of considerable interest for clinical trials of inhibitors of the tyrosine kinase of EGFR.[4,15] The kinase inhibitors erlotinib (Tarceva, OSI Pharmaceuticals) and gefitinib (Iressa, AstraZeneca) have been studied most extensively in clinical trials.[15-18]

Somatic mutations in the region of *EGFR* that encodes the tyrosine kinase domain of the receptor (exons 18 through 21) have been identified in lung cancer,[19-21] and many studies suggest that they can be used to predict responsiveness to gefitinib and erlotinib.[19-30] Such mutations occur more frequently in patients with adenocarcinoma, women, Asians, and patients who have never smoked. Whether *EGFR* mutations are more accurate predictors of responsiveness to inhibitors of *EGFR* than are these clinical factors has not been established. The effect of an *EGFR* mutation on prognosis and survival after treatment with an EGFR inhibitor is unclear, since studies to date have not included an untreated control group. The presence of mutations that affect extracellular domains of the receptor[31] does not predict outcome, and it is not known whether some of the newly identified mutations of the tyrosine kinase region are superior to others in predicting responsiveness. Stephens et al.[32] recently identified mutations in the kinase domain of the gene for the growth factor receptor HER2 in 4 percent of non–small-cell lung cancer tumors (10 percent of adenocarcinomas), but their clinical significance is unknown.

The National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) BR.21 placebo-controlled study demonstrated a survival advantage for patients with non–small-cell lung cancer who received erlotinib after other treatments had failed.[33] Women, Asians, patients with adenocarcinoma, and patients who had never smoked were more likely than other patients to have a response to erlotinib; however, those who had never smoked had a significant survival benefit from erlotinib. To clarify the role of EGFR in the outcome of non–small-cell lung cancer, we evaluated the expression of EGFR protein, the number of copies of *EGFR*, and mutation status of the gene in a subgroup of patients in the BR.21 study.

METHODS

CLINICAL STUDY

The BR.21 study was a phase 3 trial of erlotinib involving patients who had had progression after standard chemotherapy for non–small-cell lung cancer.[33] Patients were randomly assigned in a 2:1 ratio to receive 150 mg of erlotinib daily (OSI Pharmaceuticals) or placebo. The primary end point was overall survival. Response[34] was a secondary end point. Separate written consent was obtained for optional tissue banking and correlative studies. All studies were designed, executed, and analyzed by the NCIC CTG; the database was maintained by the NCIC CTG; and the manuscript was written by members of the NCIC CTG. OSI Pharmaceuticals reviewed the final manuscript.

PATHOLOGY, ANALYSIS OF EXPRESSION OF EGFR, AND MOLECULAR ANALYSES

Interpretation of all EGFR analyses was blinded with respect to clinical response and demographic information. Paraffin blocks or 10 to 20 unstained slides were collected from diagnostic or resection specimens. The presence of adequate tumor tissue was verified by the study pathologist. The expression of EGFR protein was determined by means of immunohistochemistry with the use of Dako EGFR PharmDx kits (DakoCytomation). When more than 10 percent of tumor cells demonstrated membranous (partial or complete) staining of any intensity, the tumor was considered positive for EGFR.

The entire 5-μm tissue section of specimens with cellularity of more than 50 percent was scraped from the slide for DNA isolation and mutational analyses. For specimens with lesser degrees of tumor cellularity or uneven distribution of tumor cells, enriched DNA was isolated from tumor cells that were microdissected from sections stained with toluidine blue (Fisher Canada) with the use of a dissecting microscope (model SZPT40, Olympus) at 40× magnification. In some cases, we used laser

Table 1. Baseline Demographic Characteristics of All Patients and the Patients Who Underwent Immunohistochemical (IHC) Analysis, Fluorescence in Situ Hybridization, and Mutational Analysis.*

Characteristic	IHC Analysis (N=325)	FISH (N=125)	Mutational Analysis (N=177)	All Patients (N=731)
Hazard ratio for death (95% CI)†	0.76 (0.59–0.97)	0.63 (0.42–0.96)	0.71 (0.50–0.99)	0.70 (0.58–0.85)
		number of patients (percent)		
Age				
<60 yr	149 (46)	49 (39)	79 (45)	332 (45)
≥60 yr	176 (54)	76 (61)	98 (55)	399 (55)
Sex				
Female	115 (35)	44 (35)	66 (37)	256 (35)
Male	210 (65)	81 (65)	111 (63)	475 (65)
Performance status				
0 or 1	203 (62)	91 (73)	127 (72)	486 (66)
2 or 3	122 (38)	34 (27)	50 (28)	245 (34)
P value	0.04	—	—	—
Histopathological subtype				
Adenocarcinoma	164 (50)	67 (54)	95 (54)	365 (50)
Other	161 (50)	58 (46)	82 (46)	366 (50)
Race or ethnic group				
Asian	20 (6)	8 (6)	12 (7)	91 (12)
Other	305 (94)	117 (94)	165 (93)	640 (88)
P value	<0.001	0.03	0.01	—
Smoking history				
Current	49 (15)	15 (12)	20 (11)	110 (15)
Former	181 (56)	74 (59)	104 (59)	404 (55)
None	68 (21)	32 (26)	42 (24)	146 (20)
Unknown	27 (8)	4 (3)	11 (6)	71 (10)
P value	—	0.02	—	—
Prior cisplatin				
Yes	301 (93)	118 (94)	166 (94)	678 (93)
No	24 (7)	7 (6)	11 (6)	53 (7)
No. of prior regimens				
1	140 (43)	44 (35)	61 (34)	364 (50)
2 or 3	185 (57)	81 (65)	116 (66)	367 (50)
P value	0.001	<0.001	<0.001	—
Best response to prior chemotherapy				
Complete or partial response	133 (41)	59 (47)	76 (43)	292 (40)
Stable disease	137 (42)	52 (42)	80 (45)	287 (39)
Progression	55 (17)	14 (11)	21 (12)	152 (21)
P value	—	0.01	0.003	—
Time from diagnosis to randomization				
≤12 mo	134 (41)	52 (42)	65 (37)	339 (46)
>12 mo	191 (59)	73 (58)	112 (63)	392 (54)
P value	0.01	—	0.003	—
Decrease in body weight				
<5%	210 (65)	84 (67)	116 (66)	486 (67)
≥5%	99 (30)	34 (27)	47 (26)	213 (29)
Data missing	16 (5)	7 (6)	14 (8)	32 (4)
P value	—	—	0.03	—

* P values reflect significance in a subgroup as compared with the entire study population. FISH and mutational analysis included only patients whose results could be evaluated. CI denotes confidence interval.
† The comparison group is the placebo group.

capture microdissection with a PixCell II System (Arcturus Bioscience). After proteinase K digestion, DNA was isolated according to the phenol–chloroform protocol. Exons 18 through 21 of the *EGFR* gene were sequentially amplified by two rounds of polymerase-chain-reaction (PCR) assays with the use of AmpliTaq Gold (Applied Biosystems) and external and internal primer sets designed by Paez et al.[20] Purified PCR products were sequenced in both directions with the use of the BigDye Terminator Cycle Sequencing Kit (version 3.1, Applied Biosystems) and an ABI Genetic Analyzer (model 3100, Applied Biosystems). Sequence data were analyzed by means of SeqScape software (version 2.1.1, Applied Biosystems), followed by manual review. Only sequence variations that were present in both directions in more than 15 percent of specimens were included in the analysis. When sufficient material was available, a second PCR assay was performed.

Fluorescence in situ hybridization (FISH) studies were performed with the use of dual-color DNA FISH probes containing the LSI EGFR (Vysis) probe specific for the *EGFR* locus (7p12) labeled with Spectrum Orange (Vysis) and the *CEP7* chromosome 7 centromere (7p11.1 through q11.1) probe labeled with Spectrum Green (Vysis). We analyzed 33 to 100 nonoverlapping tumor-cell nuclei to determine the number of red (*EGFR*) and green (*CEP7*) signals observed as well as the pattern of distribution of signals. We also determined the number of copies of *EGFR* and classified them according to the six FISH categories defined by Cappuzzo et al.[22] Samples with a high number of copies of *EGFR* (high degrees of polysomy or amplification) were considered to be FISH-positive.

STATISTICAL ANALYSIS

Exploratory analyses were performed to characterize the relationships between EGFR status and baseline clinical characteristics and outcomes with the use of the chi-square or Fisher's exact test. Cox regression models were used to correlate outcomes according to the time to an event, and logistic-regression models were used to correlate response to EGFR status and other baseline factors. All 731 randomized patients were included in survival analyses, and all 427 patients with measurable disease who were treated with erlotinib were included in analyses of the response. All reported P values are two-sided.

RESULTS

PATIENTS

Between August 2001 and January 2003, 731 patients were enrolled: 488 were assigned to receive erlotinib, and 243 to receive placebo. Biopsy tissue was available from 532 patients, but only 472 patients (313 in the erlotinib group and 159 in the placebo group) consented to tissue banking. A tissue sample adequate for at least one analysis was available from 328 patients (212 in the erlotinib group and 116 in the placebo group). The characteristics of samples that, after pathological review, contained sufficient tumor cells to attempt mutational and FISH analyses are described in Table 1 of the Supplementary Appendix (available with the full text of this article at www.nejm.org).

Table 1 shows the baseline characteristics of all patients and those who underwent EGFR testing. Although there were significant differences in some characteristics between patients who underwent various EGFR tests and the study population as a whole, the benefit of erlotinib, as compared with placebo, was similar in both the entire study group (hazard ratio for death, 0.70; P<0.001) (Fig. 1A) and the subgroup that underwent at least one EGFR analysis (hazard ratio, 0.76; P=0.03) (Fig. 1B).

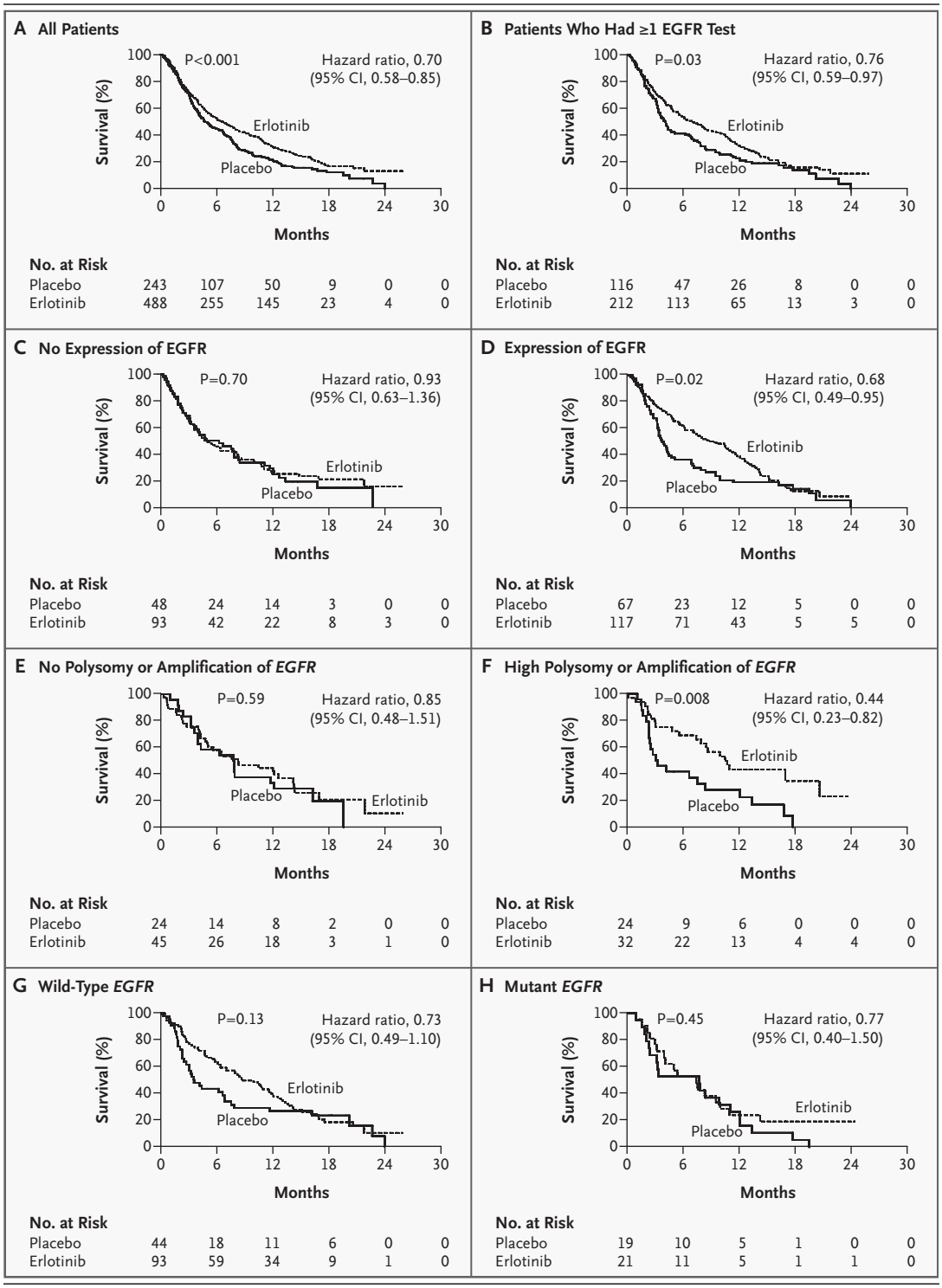
EXPRESSION OF EGFR, NUMBER OF COPIES OF *EGFR*, AND *EGFR* MUTATIONS

Among 325 tumors subjected to immunohistochemical analysis (Fig. 2 and Table 2), 184 (57 per-

Figure 1 (facing page). Kaplan–Meier Estimates of Survival.

Panel A shows the results for all 731 study patients. Panel B shows the results for the 328 patients who had at least one EGFR analysis. Panel C shows the results for patients who did not have expression of EGFR on immunohistochemical analysis (less than 10 percent of tumor cells had membranous staining). Panel D shows the results for patients who had expression of EGFR on immunohistochemical analysis (10 percent or more of tumor cells had membranous staining). Panel E shows the results for patients who did not have *EGFR* amplification or high polysomy (four or more copies of *EGFR* in at least 40 percent of cells). Panel F shows the results for patients who had *EGFR* amplification or high polysomy. Panel G shows the results for patients who had wild-type *EGFR*. Panel H shows the results for patients who had *EGFR* mutations. P values were calculated with the use of a stratified log-rank test in Panel A and the log-rank test in Panels B through H.

cent) were EGFR-positive (50 percent of adenocarcinoma samples and 63 percent of samples of other types of tumors). FISH was attempted in 221 tumors and was successful in 125 (57 percent) (Fig. 2 and Table 2). Of these, 45 percent had high polysomy or amplification (48 percent of adenocarcinoma samples and 41 percent of samples of other types of tumors).



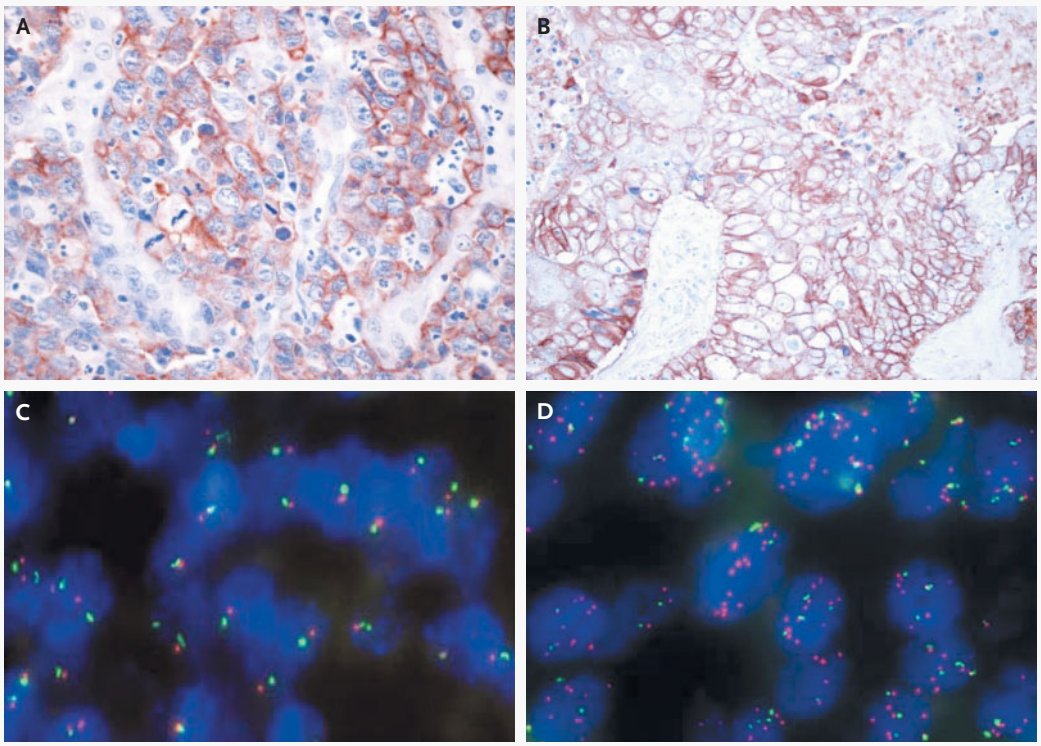


Figure 2. Expression of EGFR Protein on Immunohistochemical Analysis (Panels A and B) and Fluorescence in Situ Hybridization Analysis of the Number of Gene Copies (Panels C and D).

Incomplete (Panel A) and complete (Panel B) membranous immunohistochemical staining are scored as positive. FISH of diploid tumor cells shows one or two red (*EGFR*) and one or two green (*CEP7*) signals in most cells (Panel C), whereas cells with amplification show an excess of red signals (Panel D).

Mutational analyses were attempted in 197 samples, 110 of which yielded sufficient DNA to amplify and sequence exons 18 through 21 (59 required microdissection). Among these 110 samples, the analysis was unsuccessful in 3 samples and exons 19 and 21 were successfully analyzed in 107 samples, 24 of which (22 percent) contained one or more mutations. The 83 samples without mutations in exons 19 and 21 were classified as wild type, although some analyses of exons 18 (6 samples) and 20 (7 samples) had failed. The remaining 87 biopsy specimens of the original 197 contained small amounts of tissue (1 to 3 mm in diameter); microdissection was required for 45, since only 20 to 30 percent of cells were malignant. These 87 samples yielded DNA that was adequate for analysis of exons 19 and 21 alone. Analysis of 17 of the specimens was considered unsuccessful because one of these exons had no mutations and the other failed to yield a definitive result. Analysis of the remaining 70 specimens revealed 17 mutations in 16 samples (23 percent). Thus, mutational analysis was successful in 177 of the 197 tumor specimens that were evaluated (90 percent).

In total, 45 mutations were found in 40 patients: 3 mutations in exon 18, 13 deletions and 8 mutations in exon 19, 5 mutations in exon 20, and 16 mutations in exon 21 (Fig. 3 and Table 2 in the Supplementary Appendix). Mutations were found in 28 percent of adenocarcinoma samples examined, 16 percent of tumor specimens of other histologic types ($P=0.05$), 24 percent of specimens from women, 22 percent of specimens from men, 31 percent of specimens from patients who had never smoked, 21 percent of specimens from patients who were current or former smokers, 50 percent of specimens from Asian patients, and 21 percent of specimens from patients in other racial or ethnic

groups (P=0.03). The presence of a mutation was not correlated with the expression of EGFR or the number of copies of *EGFR*.

RESPONSIVENESS TO ERLOTINIB

Univariate analysis of data from 427 patients who could be evaluated and who had received erlotinib (Table 3) showed that the following clinical features were significantly associated with responsiveness to erlotinib: female sex (P=0.007), Asian origin (P=0.02), never having smoked (P<0.001), adenocarcinoma (P<0.001), and polysomy or amplification of *EGFR* (P=0.03). Mutational status had no significant association with responsiveness: 7 percent of those with wild-type *EGFR* had a response, as compared with 16 percent of those with an *EGFR* mutation (P=0.37). Multiple logistic-regression analyses revealed that only never having smoked (P<0.001), adenocarcinoma (P=0.01), and expression of EGFR (P=0.03) were associated with responsiveness in patients with samples that underwent immunohistochemical analysis and that never having smoked (P<0.001), adenocarcinoma (P=0.02), and polysomy or amplification of *EGFR* (P=0.04) were associated with responsiveness in patients with samples subjected to FISH.

SURVIVAL

Among the patients in both the placebo and erlotinib groups who had at least one EGFR test, the status of EGFR protein expression, the number of copies of *EGFR*, and *EGFR* mutational status were not significantly associated with survival in multivariate analysis, nor were there significant interactions between treatment groups with respect to the status of protein expression (P=0.25), the number of copies of *EGFR* (P=0.10), or mutational status (P=0.97). Survival among patients with expression of EGFR was longer in the erlotinib group than in the placebo group (hazard ratio for death, 0.68; 95 percent confidence interval, 0.49 to 0.95; P=0.02) (Table 3 and Fig. 1D), but there was no survival advantage among patients with EGFR-negative tumors (hazard ratio, 0.93; 95 percent confidence interval, 0.63 to 1.36; P=0.70) (Table 3 and Fig. 1C).

Among patients with polysomy or amplification of *EGFR*, survival was significantly longer among those who received erlotinib than among those who received placebo (hazard ratio for death, 0.44; 95 percent confidence interval, 0.23 to 0.82; P=0.008) (Table 3 and Fig. 1F), but there was no significant difference in the length of survival between groups among patients with FISH-negative tumors (hazard ratio, 0.85; 95 percent confidence interval, 0.48 to 1.51; P=0.59) (Table 3 and Fig. 1E). Mutational status had no significant effect on survival. The risk of death did not differ significantly among patients with *EGFR* mutations (even Asian patients) who received erlotinib, as compared with such patients who received placebo (hazard ratio for death, 0.77; 95 percent confidence interval, 0.40 to 1.50; P=0.54) (Fig. 1H), or among patients with wild-type *EGFR*

Table 2. Summary of the Results of EGFR Analyses and Characteristics of the Patients with *EGFR* Mutations in Tumor Specimens.

Variable	Value
Immunohistochemical analysis of EGFR protein expression — no. of samples (%)	325
Negative (<10% of tumor cells positive for membranous staining)	141 (43)
Positive (≥10% of tumor cells positive for membranous staining)	184 (57)
FISH to determine no. of copies of *EGFR* — no. of samples (%)	125
Disomy (≤2 gene copies in >90% of cells)	13 (10)
Low trisomy (3 gene copies in 10–39% of cells)	23 (18)
High trisomy (3 gene copies in ≥40% of cells)	3 (2)
Low polysomy (≥4 gene copies in 11–39% of cells)	30 (24)
High polysomy (≥4 gene copies in ≥40% of cells)	42 (34)
Amplification (gene:chromosome ≥2 or ≥15 gene copies per cell in ≥10% of cells)	14 (11)
Sequencing to identify *EGFR* mutations*	177
Wild type (no deletions or mutations detected in exons 18–21) — no. (%)	137 (77)
Mutations — no. (%)	40 (23)
Type of mutation — no. of mutations (%)	45
Exon 19 deletion	13 (29)
Exon 21 L858R	8 (18)
Other variant	24 (53)
Characteristics of the patients with *EGFR* mutations	
Geographic region — no. of patients/total no. (%)	
North America	12/56 (21)
South America	18/78 (23)
Australia or New Zealand	1/9 (11)
Asia	5/14 (36)
Continental Europe	4/20 (20)
Race or ethnic group — no. of patients/total no. (%)	
Asian	6/12 (50)
Other	34/165 (21)

* A total of 45 mutations were identified in 40 patients.

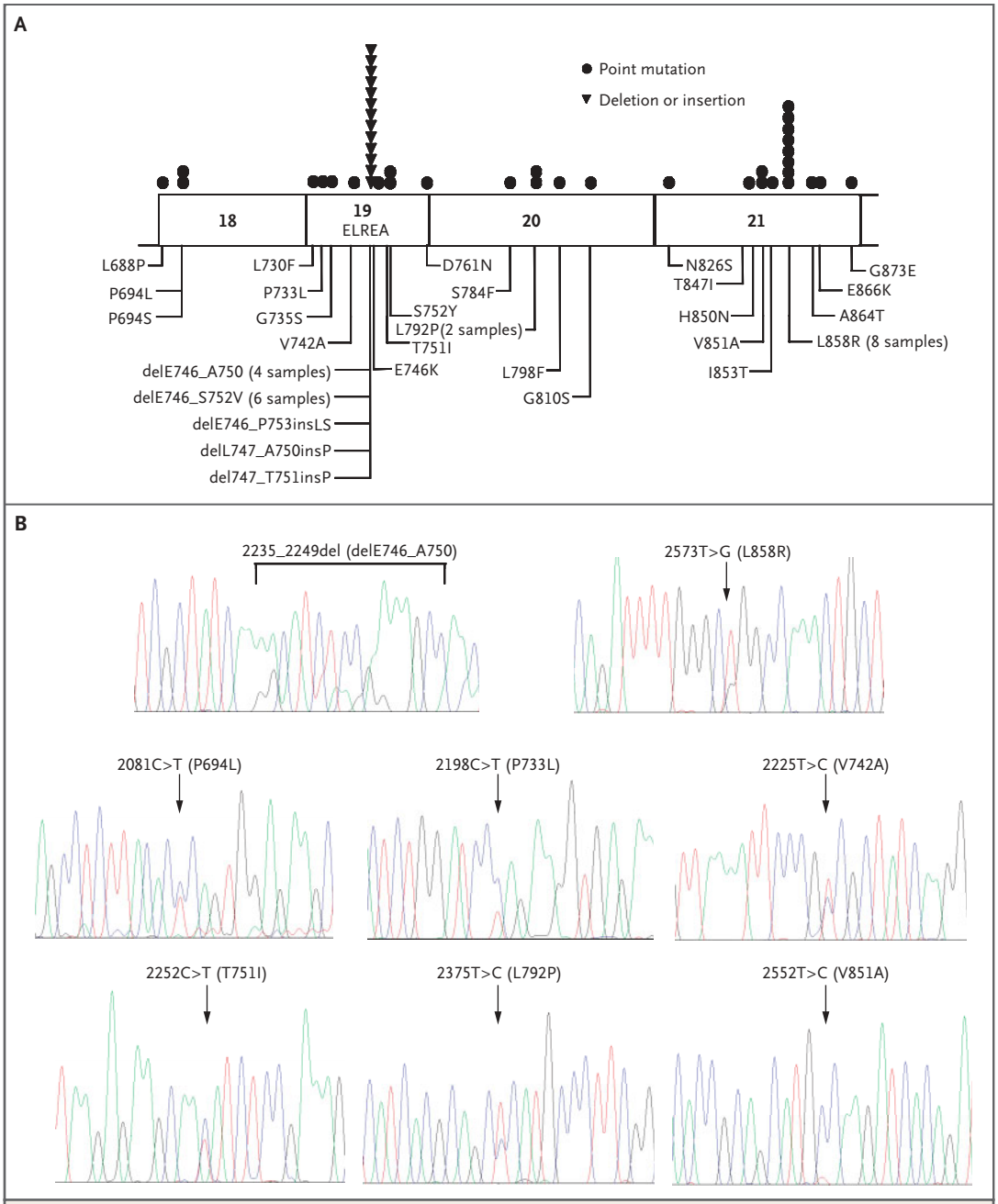


Figure 3. Mutations in the *EGFR* Gene.

Panel A shows the distribution of 45 mutations in exons 18 through 21 of the *EGFR* gene. Panel B shows representative sequencing results of typical mutations (delE746_A750 and 2573T>G) and novel variant mutations. For the specimen with the V851A mutation, the minor peak represents the wild-type T nucleotide. All mutations were confirmed bidirectionally with forward and reverse sequencing.

who received erlotinib, as compared with such patients who received placebo (hazard ratio for death, 0.73; 95 percent confidence interval, 0.49 to 1.10; P=0.13) (Fig. 1G).

Twenty-one mutations (in 20 patients) were previously described deletions in exon 19 or the L858R mutation in exon 21. There was no significant difference in survival associated with erlotinib thera-

Table 3. Analysis of Responses and Survival.*

Factor	No. of Patients	No. Who Could Be Evaluated	No. of Responses (%)	P Value	Hazard Ratio for Death (95% CI)†	P Value
Entire study group	731	427	38 (9)	—	0.70 (0.58–0.85)	<0.001
Sex				0.007		
Male	475	281	17 (6)		0.76 (0.62–0.94)	0.01
Female	256	146	21 (14)		0.80 (0.59–1.07)	0.13
Histopathological subtype				<0.001		
Adenocarcinoma	365	209	29 (14)		0.72 (0.56–0.92)	0.008
Other	366	218	9 (4)		0.81 (0.64–1.02)	0.07
Smoking history				<0.001		
Current or former	545	311	12 (4)		0.87 (0.71–1.05)	0.14
None	146	93	23 (25)		0.42 (0.28–0.64)	<0.001
Unknown	40	23	3 (13)		1.09 (0.54–2.22)	0.80
Race or ethnic group				0.02		
Asian	91	53	10 (19)		0.61 (0.37–1.03)	0.06
Other	640	374	28 (7)		0.79 (0.66–0.95)	0.01
Results of EGFR IHC analysis	325			0.1		
Positive‡	184	106	12 (11)		0.68 (0.49–0.95)	0.02
Negative	141	80	3 (4)		0.93 (0.63–1.36)	0.70
FISH status	125			0.03		
Not amplified	69	41	1 (2)		0.85 (0.48–1.51)	0.59
Amplified§	56	25	5 (20)		0.44 (0.23–0.82)	0.008
Mutational status	170			0.37		
Wild type	137	81	6 (7)		0.73 (0.49–1.10)	0.13
Mutation	40	19	3 (16)		0.77 (0.40–1.50)	0.45

* Response was assessed according to the Response Criteria in Solid Tumors (RECIST criteria)[34] in patients who had one or more lesions that could be evaluated and at least one follow-up radiologic examination. CI denotes confidence interval, and IHC immunohistochemical.
† The comparison group is the placebo group.
‡ A positive result was one in which 10 percent or more of cells had membranous staining.
§ Results include findings of high polysomy and amplification, as defined in Table 2.

py, as compared with placebo, among patients with the classic exon 19 deletions or the exon 21 L858R mutation (hazard ratio for death, 0.65; 95 percent confidence interval, 0.24 to 1.75; P=0.39) and those with only novel mutations (hazard ratio, 0.67; 95 percent confidence interval, 0.26 to 1.75; P=0.41) (Fig. 1 of the Supplementary Appendix).

In multivariate Cox regression analysis, treatment with erlotinib, as compared with placebo, remained significantly associated with longer survival (P=0.001). In the entire group, Asian patients (P=0.01), patients who had never smoked (P<0.001), patients who lost less than 5 percent of their body weight (P=0.03), patients with a performance status of 0 or 1 (P<0.001), patients who had not previously received cisplatin (P=0.04), and patients who enrolled in the study more than 12 months after receiving a diagnosis of non–small-cell lung cancer (P<0.001) survived longest.

DISCUSSION

Phase 2 studies have shown that female sex, adenocarcinoma, Asian origin, and never having smoked are associated with responsiveness of non–small-cell lung cancer to erlotinib or gefitinib.[16-18,35-37] We confirmed these associations.

The expression of EGFR protein on immuno-

histochemistry has not been a reliable predictor of responsiveness in most studies of EGFR inhibitors.[18,37,38] In our trial, 57 percent of the patients who were tested had tumors that expressed EGFR, and on multivariate analysis, their response rate was higher than that of patients with EGFR-negative tumors (11 percent vs. 4 percent). The expression of EGFR is often associated with polysomy or amplification of *EGFR*.[12] We found that the response rate was significantly higher among patients with tumors with high polysomy or amplification of *EGFR* than among those without this characteristic (20 percent vs. 2 percent). Cappuzzo et al.[22] found that an increased number of copies of *EGFR* was a stronger predictor of response than was the expression of EGFR.

Several groups have found mutations in the *EGFR* tyrosine kinase domain (exons 18 through 21) that sensitize tumor cells to the effects of erlotinib or gefitinib and appear to be associated with responsiveness to these drugs.[19-30] The reported mutations are in-frame deletions, with or without insertions in exon 19, and missense point mutations, mainly in exon 21. The prevalence of mutations varies, ranging from 20 to 40 percent in Asian countries (Taiwan, Korea, and Japan)[23,24,26-28] and from 5 to 19 percent in Italy.[22,25] We identified 45 mutations (13 deletions and 32 point mutations) in 40 patients. The 23 percent prevalence of mutations in our population (40 of 177 patients) is similar to that reported by Shigematsu et al., who analyzed 617 tumors from patients from various regions of the world and found the highest rates among Asian patients.[27] The most common mutations in their study were short deletions in exon 19, corresponding to the region between amino acids 746 and 753 in *EGFR*, and the exon 21 L858R mutation. They also identified point mutations that had not previously been reported. Huang et al.[24] reported 10 new mutations among 117 tumors; 5 were found in 16 tumors that had been embedded in paraffin. Among the approximately 2400 reported analyses for *EGFR* mutations,[19-28] only about 10 percent were performed in formalin-fixed paraffin-embedded specimens, and few routinely used microdissection to increase the number of tumor cells in a given sample.[22,24] The routine application of microdissection to enrich tumor-cell DNA may increase the rate of detection of new mutations. Multiple (two or three) mutations have been identified in individual tumors.[21,23,24,27,29]

In our study, 21 mutations were either deletions in exon 19 or the exon 21 L858R mutation and 24 were novel mutations. In keeping with our results, increasing numbers of novel mutations are being reported in lung cancers and other types of tumors.[23,24,39,40] Among the 24 novel mutations we identified, 1 involves a previously reported codon (V851) but a change in a different amino acid (V851A rather than V851I),[22] and several have also been identified recently by other groups[39] (and unpublished data). In 21 of our patients with such mutations, sufficient DNA was available for reanalysis, and thus, we used independent PCR to confirm the novel sequence in one specimen (D761N). No information on the functional significance of these mutations is available, and none of these mutations have been reported as polymorphisms. A more important point is that no significant difference in survival was associated with erlotinib therapy, as compared with placebo, among patients with the classic exon 19 deletions or the exon 21 L858R mutation (hazard ratio for death, 0.65; 95 percent confidence interval, 0.24 to 1.75; P=0.39) and those with only novel mutations (hazard ratio, 0.67; 95 percent confidence interval, 0.26 to 1.75; P=0.41) (Fig. 1 of the Supplementary Appendix).

The clinical characteristics of our patients with mutations were similar to those of patients in published studies, with a preponderance of female patients, patients with adenocarcinoma, nonsmoking patients, and Asian patients,[19-28] but we also identified mutations in other subgroups of patients. As in other reports, in our study, the response rate among patients with mutations was more than twice that among patients with wild-type *EGFR*, although the difference was not significant, perhaps because the number of responses was small. The presence of a mutation was not more likely to be associated with responsiveness than were other clinical characteristics.

A meta-analysis[14] has suggested that in patients with non–small-cell lung cancer who are not receiving EGFR-inhibitor therapy, expression of EGFR is not a strong prognostic factor for survival. Our findings were similar, but whether the expression of EGFR is associated with responsiveness to erlotinib or to a differential effect of erlotinib on survival requires further exploration.

Cappuzzo et al.[22] reported that only the number of copies of *EGFR* was significantly related to survival in a multivariate analysis of patients who were treated with gefitinib. In our study, however,

the number of copies of *EGFR* was not a significant prognostic factor in multivariate analysis.

The view that patients with wild-type tumors would not benefit from treatment with EGFR inhibitors and thus should not receive these agents was reinforced by reports that patients with *EGFR* mutations in their tumor cells survived longer than patients without such mutations.[26,28,30] However, none of these studies included an untreated control group, and thus, they were unable to determine whether this finding was due to a differential effect of treatment on tumors with mutations or to the indolent behavior of tumors with mutations. Although there was a higher rate of response among the small number of patients with mutations than among those with wild-type *EGFR* in our study, the presence of a mutation was not associated with the survival benefit of erlotinib therapy. The benefit from erlotinib, as compared with placebo, among patients with wild-type *EGFR* (hazard ratio for death, 0.73) was similar to that observed in the population as a whole (hazard ratio, 0.70).

In summary, multivariate analysis revealed that expression of EGFR and an increased number of copies of *EGFR*, but not mutations in *EGFR*, were associated with responsiveness to erlotinib but not with increased survival. Our results suggest that mutational analysis is not necessary to identify patients in whom treatment with EGFR inhibitors is appropriate.

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ORIGINAL ARTICLE

Single-Chamber versus Dual-Chamber Pacing for High-Grade Atrioventricular Block

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ABSTRACT

BACKGROUND

In the treatment of atrioventricular block, dual-chamber cardiac pacing is thought to confer a clinical benefit as compared with single-chamber ventricular pacing, but the supporting evidence is mainly from retrospective studies. Uncertainty persists regarding the true benefits of dual-chamber pacing, particularly in the elderly, in whom it is used less often than in younger patients.

METHODS

In a multicenter, randomized, parallel-group trial, 2021 patients 70 years of age or older who were undergoing their first pacemaker implant for high-grade atrioventricular block were randomly assigned to receive a single-chamber ventricular pacemaker (1009 patients) or a dual-chamber pacemaker (1012 patients). In the single-chamber group, patients were randomly assigned to receive either fixed-rate pacing (504 patients) or rate-adaptive pacing (505 patients). The primary outcome was death from all causes. Secondary outcomes included atrial fibrillation, heart failure, and a composite of stroke, transient ischemic attack, or other thromboembolism.

RESULTS

The median follow-up period was 4.6 years for mortality and 3 years for other cardiovascular events. The mean annual mortality rate was 7.2 percent in the single-chamber group and 7.4 percent in the dual-chamber group (hazard ratio, 0.96; 95 percent confidence interval, 0.83 to 1.11). We found no significant differences between the group with single-chamber pacing and that with dual-chamber pacing in the rates of atrial fibrillation, heart failure, or a composite of stroke, transient ischemic attack, or other thromboembolism.

CONCLUSIONS

In elderly patients with high-grade atrioventricular block, the pacing mode does not influence the rate of death from all causes during the first five years or the incidence of cardiovascular events during the first three years after implantation of a pacemaker.

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Cardiac pacing is the established treatment for high-grade atrioventricular block, but the appropriate pacing mode remains the subject of debate.[1] Single-chamber ventricular pacing prevents bradycardia and death from ventricular standstill, but dual-chamber pacing better emulates normal cardiac physiology by restoring atrioventricular synchrony and matching the ventricular pacing rate to the sinus rate. As a result, dual-chamber pacing, as compared with single-chamber ventricular pacing, improves hemodynamic function,[2-4] but the clinical benefit is uncertain.

Nonrandomized studies suggest that dual-chamber pacing is associated with a lower incidence of atrial fibrillation, stroke, and heart failure than is single-chamber pacing.[5] There is also evidence of improved survival, but the data are confounded by selection bias, because of the preferential use of dual-chamber pacing in younger patients with fewer coexisting illnesses.[6] Some current guidelines recommend dual-chamber pacing unless atrial fibrillation is present,[7] but the limitations of the supporting data have led to questions about the guidelines[8] and the apparent underuse of dual-chamber pacing, particularly in the elderly.[9,10]

The United Kingdom Pacing and Cardiovascular Events (UKPACE) trial compared the clinical benefits of single-chamber ventricular pacing and dual-chamber pacing in elderly patients with atrioventricular block.[10]

METHODS

The UKPACE trial was a randomized, parallel-group trial conducted in 46 centers in the United Kingdom, representing a wide range of experience among centers and operators. The trial was approved by the North West Multi-Centre Research Ethics Committee and the local research ethics committee for each center. All patients provided written informed consent.

PATIENT SELECTION

Patients were recruited from August 22, 1995, to September 24, 1999. All new pacemaker implantations were registered, and trial eligibility was recorded. Eligible patients were 70 years of age or older and scheduled for their first pacemaker implantations for high-grade (i.e., second-degree or complete) atrioventricular block. Exclusion criteria included chronic established atrial fibrillation, New York Heart Association (NYHA) class IV heart failure, advanced cognitive dysfunction, total immobility, and advanced cancer (life expectancy of less than one year). Patients with persistent atrial fibrillation of less than three months' duration were eligible if they had undergone cardioversion and had normal sinus rhythm at enrollment.

DATA COLLECTION, RANDOMIZATION, IMPLANTATION, AND PROGRAMMING

Baseline demographic and clinical characteristics of the patients were recorded by physicians, nurses, or cardiac technicians. Patients were randomly assigned, up to 24 hours before the scheduled implantation, to receive either a fixed-rate or rate-adaptive single-chamber ventricular pacing system or a dual-chamber system (with or without sensor-modulated rate adaptation). Within the single-chamber group, assignment to fixed-rate or rate-adaptive pacing was randomly determined. Randomization was performed with the use of a 24-hour automated telephone-based system, linked to a computer at the data center, with a dynamic balancing algorithm that stratified patients by center and age (<80 years or ≥80 years). Implantation was performed according to the operator's usual practice.

The use of sensor-based rate adaptation in the dual-chamber group and the programming of variables other than mode were determined by the investigator. Suggested settings for dual-chamber pacemakers were an atrioventricular delay of 150 msec, rate-adaptive atrioventricular shortening to 75 msec, and lower and upper rate limits of 60 beats per minute and 125 beats per minute, respectively. For rate-adaptive single-chamber pacemakers, the suggested lower and upper rate limits were 70 beats per minute and 125 beats per minute, respectively. For fixed-rate single-chamber pacemakers, a rate of 70 beats per minute was suggested. The operators and patients were not blinded to the type of pacing system used or the programming of the system.

END POINT AND OUTCOMES

The primary end point was death from all causes. Prespecified cardiovascular events included atrial fibrillation (defined as an episode, with or without symptoms, lasting 15 minutes or more and verified by electrocardiography), new or significantly worsening heart failure, a composite of stroke, transient ischemic attack, or other thromboembolism, revision of the pacing system, new-onset angina

or newly diagnosed ischemic heart disease, and myocardial infarction. Crossover, in the event of suspected intolerance of the pacing mode, was at the discretion of the investigator.

Patients were followed for a minimum of 3 years, with scheduled visits at 1, 4, 10, 16, and 36 months, at which the pacemaker function was assessed and outcome events were recorded. Patients were given a diary in which the details of any medical contacts between their follow-up visits were to be recorded. The identity of enrolled patients was given to the U.K. Office for National Statistics, which provided automatic notification of registered deaths. Mortality data were censored on September 24, 2002. Data for other cardiovascular events were censored at the actual or intended date of the 36-month visit. If the visit was missed, outcome data were sought through a review of clinical records or through contact with the patient's family doctor. Deaths and specified cardiovascular events were adjudicated and classified by an independent committee on end points and events, with members unaware of the pacing modes. Deaths were classified as due to cardiovascular or noncardiovascular causes and treated as the result of cardiovascular causes if attributed to old age or if the cause was unclear. Safety was monitored by an independent data-monitoring committee.

STATISTICAL ANALYSIS

The database was maintained and analyzed by an independent data-management group (Nottingham Clinical Research Group, Nottingham, United Kingdom). The trial was designed to have a power of at least 90 percent to detect a 25 percent reduction in the primary end point, with a target recruitment of 2000 patients. This assumed an annual mortality rate of 8 percent in the single-chamber group, with allowance for a 6 percent crossover from single-chamber to dual-chamber pacing. More deaths were reported than anticipated, giving the study 95 percent power to detect a 25 percent reduction in mortality.

Differences in the proportion of ventricular beats that were paced were compared with the use of the Wilcoxon rank-sum test. Cumulative-event rates were calculated according to the Kaplan–Meier method,[11] and differences between the groups were assessed with the use of the Cox proportional-hazards model,[12] with adjustment for the baseline covariates of age (≤80 years vs. >80 years), sex, and NYHA class (I or II vs. III or IV). Relative risk was calculated after adjustment for age, sex, and NYHA class at baseline and expressed as hazard ratios with 95 percent confidence intervals. The primary analysis was the comparison of single-chamber and dual-chamber pacing. A prespecified secondary analysis involved the separate comparisons of fixed-rate and rate-adaptive single-chamber pacing with dual-chamber pacing. The impact of baseline covariates on the hazard ratio for the primary end point was assessed with the use of the log-rank test.[13] The incidence of procedural and predischarge complications in the two groups was compared with the use of the chi-square test. All statistical tests were two-tailed. Analysis was performed on the basis of the intention-to-treat principle.

The study was designed by Drs. Toff, Skehan, and Camm and David de Bono in collaboration with the trial steering committee and other participating investigators (see the Appendix) and funded by the Medical Research Council of the United Kingdom. Pacemakers were purchased according to normal practice, but in centers that had previously implanted less than 50 percent dual-chamber pacing systems, additional hardware costs were met by a subsidy from pacemaker manufacturers and suppliers under the terms of an agreement with the International Association of Prosthesis Manufacturers. No pacemaker manufacturer or supplier had any involvement in the study design, data collection, analysis of results, or writing of the manuscript. The manuscript was written by the principal investigators and the first draft reviewed by the trial steering committee and other participating investigators.

RESULTS

SCREENING

During the recruitment period, 16,375 patients received a first pacemaker implantation at the participating centers. Of these, 5308 (32.4 percent) had high-grade atrioventricular block and were 70 years of age or more. Exclusion criteria were documented in 945 patients, leaving 4363 eligible for the study, of whom 1972 (45.2 percent) were enrolled. The remaining patients were excluded because the patient declined to participate (13.2 percent), because the physician declined (15.0 percent), or for other or unstated reasons (26.6 percent). Forty-nine patients who were enrolled were not included in the registry either because they did not receive a pacemaker or because registry data were missing for the relevant period.

Table 1. Baseline Demographic and Clinical Characteristics of the Patients.*

Characteristic	Single-Chamber Fixed-Rate Pacing Group (N=504)	Single-Chamber Rate-Adaptive Pacing Group (N=505)	Dual-Chamber Pacing Group (N=1012)
Age (yr)	79.8±6.0	80.1±6.1	79.9±6.1
Male sex (%)	56.0	57.4	57.2
White race (%)†	95.2	95.2	96.5
New York Heart Association functional class (%)			
I	26.4	27.5	30.1
II	43.4	45.0	42.1
III	25.6	23.0	24.0
IV	1.0	2.2	0.9
Unknown	3.6	2.4	2.9
Cardiothoracic ratio on chest radiograph (%)	55.7±9.8	55.9±10.6	55.6±11.8
Primary electrocardiographic indication for implantation (%)			
Second-degree atrioventricular block	26.2	25.1	26.6
Complete atrioventricular block	73.2	74.3	72.9
Other or unknown	0.6	0.6	0.5
Presenting bradycardia (%)			
Intermittent	38.7	38.2	38.0
Constant	60.7	61.2	61.6
Unknown	0.6	0.6	0.4
Symptoms of bradycardia (%)			
Symptomatic	79.2	81.4	84.7
Asymptomatic	20.2	18.0	15.0
Unknown	0.6	0.6	0.3
Medical history (%)			
Hypertension	30.8	32.1	35.7
Diabetes	9.9	11.5	13.5
Angina	20.8	22.0	22.7
Prior myocardial infarction	15.5	15.4	12.8
Prior heart failure	15.1	16.0	16.0
Cardiac surgery	3.2	4.0	5.3
Percutaneous coronary angioplasty	0.6	0.8	0.5
Paroxysmal atrial fibrillation	3.6	3.8	4.6
Other arrhythmia	10.9	10.7	11.5
Stroke	6.3	5.9	5.4
Prior transient ischemic attack	4.6	7.5	3.9

TREATMENT ASSIGNMENT

Of 2021 patients enrolled, 1009 were randomly assigned to receive single-chamber pacing and 1012 to receive dual-chamber pacing. In the single-chamber group, 504 were assigned to fixed-rate pacing and 505 to rate-adaptive pacing. Of those assigned to single-chamber pacing, 99.4 percent received a single-chamber system, 0.2 percent received a dual-chamber system, and 0.4 percent received no pacemaker. Of those assigned to dual-chamber pacing, 95.8 percent received a dual-chamber system, 3.6 percent received a single-chamber system, and 0.6 percent received no pacemaker.

At hospital discharge, the programmed mode

Table 1. (Continued.)

Characteristic	Single-Chamber Fixed-Rate Pacing Group (N=504)	Single-Chamber Rate-Adaptive Pacing Group (N=505)	Dual-Chamber Pacing Group (N=1012)
Medication at randomization (%)			
Aspirin	38.7	39.4	42.2
Warfarin or other anticoagulant	5.6	5.9	5.6
Angiotensin-converting–enzyme inhibitor	18.7	18.4	17.8
Diuretic	44.6	47.7	49.1
Nitrate or other vasodilator	17.7	18.2	18.8
Beta-blocker	8.5	5.5	6.9
Calcium-channel blocker	13.1	14.5	17.2
Digoxin	1.4	1.4	1.9
Other antiarrhythmic agent	2.4	2.0	2.0
Lipid-lowering agent	2.6	3.8	2.9
Oral hypoglycemic agent	4.0	6.3	6.7
Insulin	2.0	2.2	2.3
Nonsteroidal antiinflammatory drug	9.1	6.5	7.0
Antidepressant	3.2	4.4	4.4

* Plus–minus values are means ±SD. Because of rounding, not all percentages total 100.
† Race was determined by the investigators.

of pacing was documented for 99 percent of the patients enrolled. At least 98.9 percent of those assigned to single-chamber pacing were being paced in a single-chamber mode, and at least 95.2 percent of those assigned to dual-chamber pacing were being paced in a dual-chamber mode. At the final follow-up or at the study end point, at least 96.9 percent of the patients assigned to single-chamber pacing were receiving this type of pacing, and at least 91.7 percent of those assigned to dual-chamber pacing were being paced in a dual-chamber mode. At the conclusion of the study, 3.1 percent of patients had crossed over from single-chamber to dual-chamber pacing, primarily because of suspected intolerance of the pacing mode.

The extent of ventricular pacing, as assessed from the pacemaker's memory at one month, was recorded for 65 percent of all patients. The median percentage of ventricular beats that were paced was 94 percent in the fixed-rate single-chamber group, 93 percent in the rate-adaptive single-chamber group, and 99 percent in the dual-chamber group. The difference in ventricular paced beats between the single-chamber group and the dual-chamber group was statistically significant (P<0.001), but the difference between fixed-rate and rate-adaptive single-chamber pacing was not (P=0.85).

BASELINE CHARACTERISTICS

The treatment groups were well balanced with regard to baseline demographic and clinical characteristics (Table 1). The mean age of the patients was 79.9 years; 57 percent were male. The indication for pacing was second-degree atrioventricular block in 26.1 percent of patients and complete atrioventricular block in 73.3 percent. Bradycardia was judged as constant in 61.3 percent of patients and intermittent in 38.2 percent. Eighty-three percent of patients were symptomatic.

OUTCOME EVENTS

The median follow-up period was 4.6 years for death and 3 years for other cardiovascular events. No patients were lost to follow-up with respect to the primary end point. Figure 1 shows Kaplan–Meier estimates of the cumulative risk of death from all causes and from cardiovascular causes in the two treatment groups, and Figure 2 shows es-

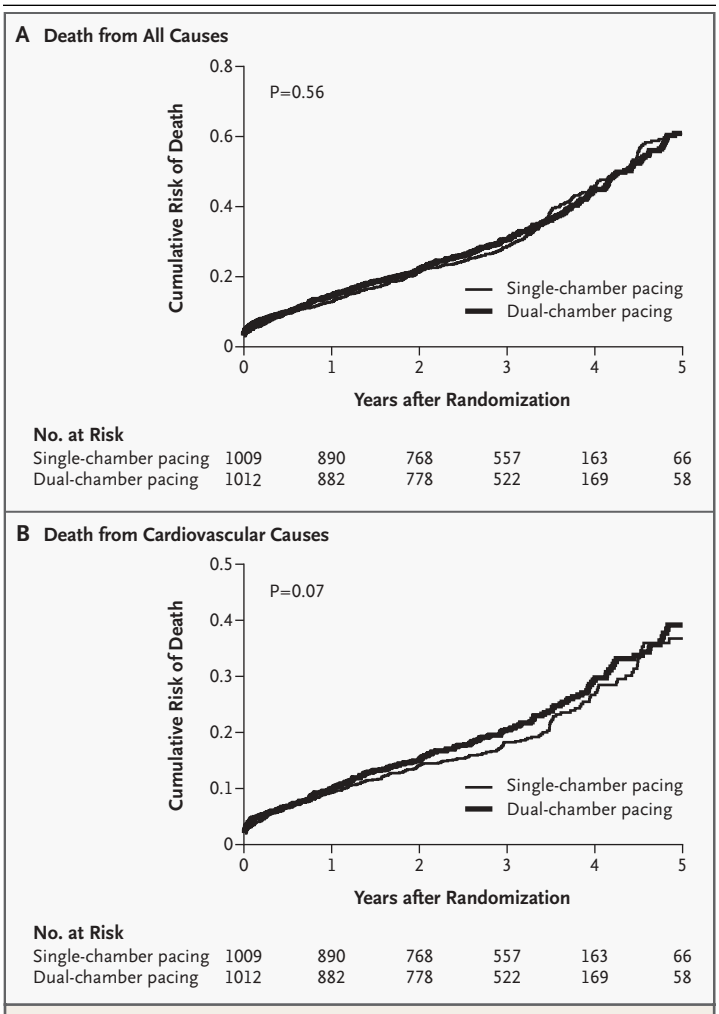


Figure 1. Cumulative Risk of Death from All Causes and from Cardiovascular Causes According to the Mode of Pacing.

timates for the risk of the specified cardiovascular events. Table 2 shows event rates and hazard ratios for death from all causes, death from cardiovascular causes, and specified cardiovascular events for the primary comparison of single-chamber pacing with dual-chamber pacing; also shown are the event rates and hazard ratios for the separate comparison of fixed-rate and rate-adaptive single-chamber pacing with dual-chamber pacing.

Mortality

The mean annual rate of death from all causes during the first five years after pacemaker implantation was 7.2 percent in the single-chamber group and 7.4 percent in the dual-chamber group (P=0.56). The mean annual rate of death due to cardiovascular causes was 3.9 percent in the single-chamber group and 4.5 percent in the dual-chamber group (P=0.07).

Atrial Fibrillation

There was a higher incidence of atrial fibrillation in the dual-chamber group during the first 18 months after pacemaker implantation but no significant difference in the mean annual event rates at 3 years, which were 3.0 percent in the single-chamber group and 2.8 percent in the dual-chamber group (P=0.74).

Stroke, Transient Ischemic Attack, or Thromboembolism

For the combined outcome of stroke, transient ischemic attack, or thromboembolism, the mean annual event rate was 2.1 percent in the single-chamber group and 1.7 percent in the dual-chamber group (P=0.20). There was a significantly higher event rate with fixed-rate single-chamber pacing (2.5 percent per year) than with dual-chamber pacing (P=0.04), but the event rate in the rate-adaptive single-chamber group (1.7 percent per year) was the same as that in the dual-chamber group (P=0.93).

Heart Failure, Angina, and Myocardial Infarction

There was no significant difference in the mean annual event rate for heart failure, which was 3.2 percent in the single-chamber group and 3.3 percent in the dual-chamber group (P=0.80). Similarly, there was no significant difference between the single-chamber and dual-chamber groups in event rates for new-onset angina or ischemic heart disease or for myocardial infarction.

SUBGROUP ANALYSIS

The subgroup analysis showed that the presence or absence of selected baseline characteristics did not affect the influence of the pacing mode on the primary end point (Fig. 3).

COMPLICATIONS

Procedural complications were more common in the dual-chamber group than in the single-chamber group (7.8 percent vs. 3.5 percent, P<0.001), as were other complications before discharge (10.4 percent vs. 6.1 percent, P<0.001). The need for therapeutic intervention was also more frequent in the dual-chamber group (8.8 percent vs. 5.6 percent, P=0.005), as were complications requiring

repeated operation before discharge (4.2 percent vs. 2.5 percent, P=0.04). The differences in complication rates were principally due to problems with the placement or stability of atrial leads.

DISCUSSION

The key finding from this randomized trial was that in elderly patients with high-grade atrioventricular block, dual-chamber pacing provided no survival advantage over single-chamber pacing. This result contrasts with the findings of nonrandomized studies that suggested there was improved survival with dual-chamber pacing, which probably reflected selection bias.[6,14]

Our trial focused on atrioventricular block in the elderly, but the absence of a survival advantage for dual-chamber pacing is consistent with the results of other trials that included younger patients and those with sinus-node disease. In the Pacemaker Selection in the Elderly (PASE) study, 407 patients 65 years of age or older with sinus-node disease or atrioventricular block were randomly assigned to receive single-chamber ventricular pacing or dual-chamber pacing.[15] The study was not powered to assess mortality, but there was no significant difference in survival or clinical outcomes during the mean follow-up period of 18 months, although the crossover rate from ventricular to dual-chamber pacing was high (26 percent).

In the Canadian Trial of Physiologic Pacing (CTOPP), 2568 patients 18 years of age or older who had sinus-node disease or atrioventricular block were randomly assigned to physiologic (atrial or dual-chamber) pacing or single-chamber ventricular pacing.[16] During the mean follow-up period of three years, there was no significant difference in the rate of death from all causes or in the primary end point of cardiovascular death or stroke. There was an 18 percent reduction in the relative risk of atrial fibrillation with dual-chamber pacing but no significant difference in the rate of hospitalization for heart failure or in the occurrence of stroke. Follow-up extended to a mean of 6.4 years showed a further reduction in the relative risk of atrial fibrillation (20.1 percent) but no other benefit from physiological pacing.[17]

The absence of a reduction in atrial fibrillation with dual-chamber pacing in the UKPACE trial contrasts with the findings of the CTOPP trial. This difference may reflect the older age of our patients, in whom any propensity toward atrial fibrillation

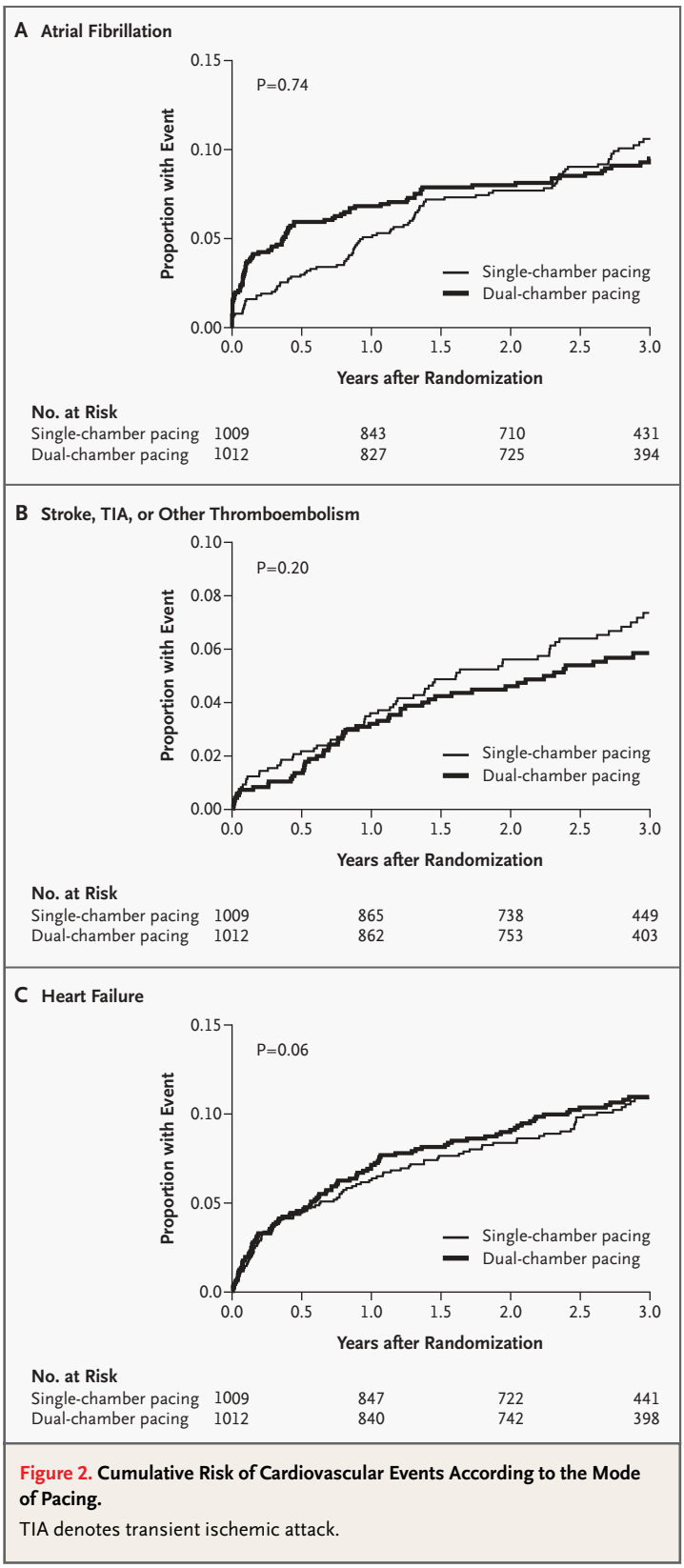


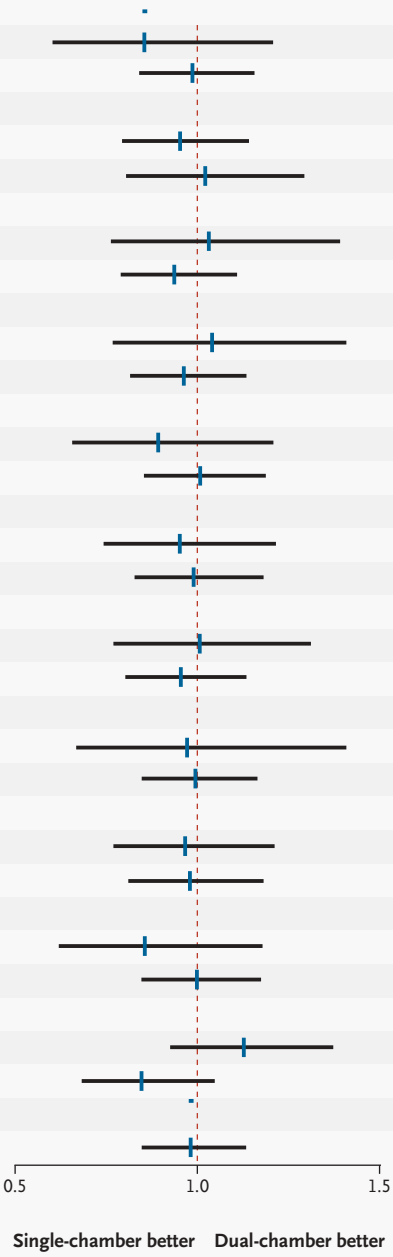
Figure 2. Cumulative Risk of Cardiovascular Events According to the Mode of Pacing.

TIA denotes transient ischemic attack.

Table 2. Event Rates and Hazard Ratios for Death from All Causes, Death from Cardiovascular Causes, and Specified Cardiovascular Outcomes in the Pacing Groups.*

	Single-Chamber Fixed-Rate Pacing Group (N=504)	Single-Chamber Rate-Adaptive Pacing Group (N=505)	Dual-Chamber Pacing Group (N=1012)	Fixed-Rate or Rate-Adaptive Single-Chamber vs. Dual-Chamber System		Fixed-Rate Single-Chamber vs. Dual-Chamber System		Rate-Adaptive Single-Chamber vs. Dual-Chamber System	
				Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
Death from all causes									
Events at 3 yr — no. (%)	125 (24.8)	128 (25.3)	277 (27.4)						
3-yr event-free survival — %	73.6	73.5	71.5						
Events at 5 yr — no. (%)	188 (37.3)	177 (35.0)	376 (37.2)						
5-yr event-free survival — %	33.5	46.0	40.0	0.96 (0.83–1.11)	0.56	1.03 (0.86–1.23)	0.74	0.89 (0.75–1.07)	0.22
Death from cardiovascular causes									
Events at 3 yr — no. (%)	73 (14.5)	82 (16.2)	179 (17.7)						
3-yr event-free survival — %	83.8	82.4	80.8						
Events at 5 yr — no. (%)	94 (18.7)	105 (20.8)	228 (22.5)						
5-yr event-free survival — %	62.7	64.4	61.2	0.84 (0.69–1.01)	0.07	0.81 (0.64–1.04)	0.10	0.86 (0.68–1.08)	0.19
Atrial fibrillation									
Events at 3 yr — no. (%)	47 (9.3)	43 (8.5)	86 (8.5)						
3-yr event-free survival — %	88.7	90.1	90.5	1.05 (0.78–1.41)	0.74	1.07 (0.75–1.53)	0.72	1.03 (0.73–1.47)	0.85
Stroke, transient ischemic attack, or other thromboembolism									
Events at 3 yr — no. (%)	38 (7.5)	25 (5.0)	51 (5.0)						
3-yr event-free survival — %	91.1	94.2	94.2	1.28 (0.88–1.86)	0.20	1.58 (1.03–2.42)	0.04†	0.98 (0.60–1.60)	0.93
Heart failure									
Events at 3 yr — no. (%)	41 (8.1)	55 (10.9)	99 (9.8)						
3-yr event-free survival — %	90.7	87.6	89.0	0.96 (0.73–1.28)	0.80	0.86 (0.60–1.23)	0.41	1.07 (0.77–1.49)	0.69
New-onset angina or ischemic heart disease									
Events at 3 yr — no. (%)	5 (1.0)	5 (1.0)	9 (0.9)						
3-yr event-free survival — %	98.9	98.9	99.0	0.89 (0.38–2.10)	0.79	0.91 (0.31–2.61)	0.86	0.88 (0.31–2.53)	0.81
Myocardial infarction									
Events at 3 yr — no. (%)	12 (2.4)	11 (2.2)	32 (3.2)						
3-yr event-free survival — %	97.3	97.5	96.4	0.72 (0.42–1.22)	0.22	0.70 (0.35–1.39)	0.31	0.73 (0.38–1.42)	0.35
Revision of pacing system									
Events at 3 yr — no. (%)	28 (5.6)	35 (6.9)	59 (5.8)						
3-yr event-free survival — %	93.5	92.0	93.1	1.12 (0.54–2.35)	0.76	1.30 (0.56–3.01)	0.54	0.95 (0.37–2.39)	0.91

* Event-free survival rates were estimated with the use of the Kaplan–Meier method.
† The result was significant at the 5 percent level.



	Hazard Ratio (95% CI)	P Value	Single-Chamber Group		Dual-Chamber Group	
			No. of Patients	Deaths	No. of Patients	Deaths
Age ≤75 yr	0.85 (0.61–1.20)	0.36	263	59	282	78
Age >75 yr	0.99 (0.85–1.15)	0.86	746	328	730	315
Male	0.95 (0.80–1.14)	0.59	572	246	579	248
Female	1.02 (0.81–1.29)	0.86	436	141	432	145
NYHA class I	1.03 (0.77–1.39)	0.84	272	85	305	91
NYHA class II–IV	0.94 (0.79–1.10)	0.43	707	281	678	287
Second-degree heart block	1.04 (0.77–1.40)	0.80	259	86	289	86
Complete heart block	0.96 (0.82–1.13)	0.64	744	299	738	304
History of chronic heart failure	0.89 (0.66–1.20)	0.45	157	83	162	90
No history of chronic heart failure	1.01 (0.86–1.18)	0.93	848	300	842	298
Known ischemic heart disease	0.95 (0.75–1.21)	0.68	293	131	291	134
No known ischemic heart disease	0.99 (0.83–1.18)	0.91	713	253	714	254
Hypertension	1.01 (0.77–1.31)	0.97	317	105	361	121
No hypertension	0.95 (0.81–1.13)	0.59	688	278	644	267
Diabetes	0.97 (0.67–1.40)	0.87	108	49	137	71
No diabetes	0.99 (0.85–1.16)	0.93	898	335	868	317
Aspirin use	0.97 (0.77–1.21)	0.76	396	149	429	165
No aspirin use	0.98 (0.82–1.18)	0.82	609	234	576	223
ACE-inhibitor therapy	0.86 (0.62–1.17)	0.33	187	78	181	79
No ACE-inhibitor therapy	1.00 (0.85–1.17)	0.98	814	302	824	309
Diuretic therapy	1.13 (0.93–1.37)	0.22	468	211	499	204
No diuretic therapy	0.85 (0.69–1.04)	0.12	536	171	506	184
Overall	0.96 (0.83–1.11)	0.56	1009	387	1012	393

Figure 3. Effect of Pacing Mode on Deaths from All Causes, According to Subgroups.

Blue vertical lines and bars indicate, for each specified subgroup, the hazard ratio for death from all causes and the associated 95 percent confidence intervals (CIs) for single-chamber pacing as compared with dual-chamber pacing. NYHA denotes New York Heart Association, and ACE angiotensin-converting enzyme.

might be less readily influenced by pacing mode. Although the CTOPP trial included patients with sinus-node disease, the reduction in atrial fibrillation with physiologic pacing was also seen in the subgroup with atrioventricular block.[18] In the CTOPP trial, the difference in the incidence of atrial fibrillation emerged only at two years. It is possible that with longer follow-up of our patients, a difference might also emerge among them.

An interesting feature in our study was the increased early incidence of atrial fibrillation in the dual-chamber group. It is possible that the atrial lead may be arrhythmogenic in the early months after implantation. Alternatively, the capacity of dual-chamber pacemakers to detect high-rate atrial episodes might have led to an earlier recognition of atrial fibrillation. This capability might also increase the overall likelihood that atrial fibrillation will be detected with these systems, potentially masking a benefit of dual-chamber pacing; however, the diagnostic requirement of an electrocardiographically verified episode lasting 15 minutes or longer would be expected to attenuate the effect of such a bias.

The finding that there was a higher rate of stroke, transient ischemic attack, or thromboembolism with fixed-rate ventricular pacing is intriguing. Conceivably, the lower heart rates associated with fixed-rate pacing might promote atrial stasis, but the finding could be due to the play of chance amid multiple comparisons and should be interpreted with caution. The observation may, however, favor the use of a rate-adaptive system if a single-chamber ventricular pacemaker is used.

Our results, supported by the PASE and CTOPP trials, suggest that the clinical benefits associated with dual-chamber pacing for atrioventricular block have been overestimated. It is perhaps counterintuitive that the hemodynamic advantages of atrioventricular synchrony do not result in greater clinical benefit. One explanation for this may be that intraventricular and interventricular dyssynchrony resulting from stimulation at the right ventricular apex might counteract the benefit of atrioventricular synchrony.[19,20] This effect might be accentuated by the higher proportion of paced ventricular beats in the dual-chamber group. It is possible that alternative strategies, such as biventricular or septal pacing, might improve hemodynamic function and clinical outcomes. This hypothesis requires testing in clinical trials.

The low crossover rate (3.1 percent) from single-chamber to dual-chamber pacing in our study was similar to that in the CTOPP trial (2.7 percent), suggesting that single-chamber pacing is well tolerated. The higher crossover rate in the PASE study (26 percent), in which randomization to mode of pacing was made by software programming rather than by assignment of hardware, may reflect variation in the incidence of pacemaker syndrome but also highlights the subjective element in the diagnosis of this syndrome.

The choice of pacing mode for elderly patients with high-grade atrioventricular block should be made on an individual basis. Over three years, dual-chamber pacing is unlikely to influence mortality or the incidence of cardiovascular events, and single-chamber ventricular pacing should be regarded as an acceptable mode. The occasional need to upgrade to dual-chamber systems because of pacing-mode intolerance must be weighed against the increased risk of complications with dual-chamber implants. In elderly patients, factors other than the mode of pacing are more likely to determine the clinical outcome.

In elderly patients with high-grade atrioventricular block, the mode of pacing has no significant influence on the rate of death from all causes in the first five years after pacemaker implantation. Fixed-rate single-chamber ventricular pacing is associated with an increased risk of stroke, transient ischemic attack, or thromboembolism, but the pacing mode does not otherwise influence the incidence of cardiovascular events in the first three years after pacemaker implantation.

Supported by a grant from the Medical Research Council of the United Kingdom (G9403190). Dr. Camm holds a chair funded by the British Heart Foundation. In centers that had previously implanted less than 50 percent dual-chamber pacing systems, additional hardware costs were met by a subsidy from the following pacemaker manufacturers and suppliers under an agreement with the International Association of Prosthesis Manufacturers: Biotronik, Cardiocare, CPI–Guidant, ELA Medical, Intermedics, Medtronic, St. Jude Medical, Sorin Biomedica, Telectronics, and Vitatron.

International Standard Randomized Controlled Trial No. 37445539.

Dr. Toff reports having received lecture fees from St. Jude Medical. Dr. Skehan reports having received consulting fees and having served on the advisory board for Medtronic. Dr. Camm reports having received consulting fees and having served on advisory boards for Guidant, St. Jude Medical, and Vitatron and having received lecture fees from St. Jude Medical and Vitatron.

This article is dedicated to the patients who participated in the trial and to the memory of Prof. David de Bono, coprincipal investigator, who died on April 29, 1999.

We are indebted to the many technical, medical, nursing, and clerical staff members who provided support at the participating centers.

APPENDIX

The following institutions and investigators participated in the UKPACE trial (total numbers of patients recruited are indicated in parentheses): *Glenfield Hospital, Leicester* (343) — J.D. Skehan, W.D. Toff, J. Villanueva, J. Kovac, K. Percy, and G.H. Broomes; *Freeman Hospital, Newcastle* (170) — R.S. Bexton, S. Henderson, and J. Cronin; *St George's Hospital, London* (154) — A.J. Camm, E. Rowland, D.E. Ward, and S. Jones; *Cardiothoracic Centre, Liverpool* (139) — R.G. Charles, J. Morland-Duff, and S. Hughes; *Queen Elizabeth Hospital, Birmingham* (120) — M.D. Gammage, D. Jones, A. Barber, and Z. Harris; *Derbyshire Royal Infirmary, Derby* (79) — A. McCance and J.E. Penrice; *Northern General Hospital, Sheffield* (72) — R.J. Bowes and R. Ecob; *Blackpool Victoria Hospital, Blackpool* (71) — G.K. Goode and A. Delaney; *Hull Royal Infirmary, Hull* (68) — G.C. Kaye and T. Houghton; *Treliske Hospital, Truro* (64) — A.K.B. Slade and F. Westwood; *University Hospital of Wales, Cardiff* (60) — M.B. Buchalter and C. McCormack; *John Radcliffe Hospital, Oxford* (52) — O.J.M. Ormerod, K. Johnston, and Y. Bashir; *UCH/Middlesex Hospitals, London* (50) — D. Holdright, R.H. Swanton, and M. Squirrell; *Queen's Medical Centre, Nottingham* (49) — R.G. Wilcox and S. Jones; *Royal Devon and Exeter Hospital, Exeter* (45) — J.W. Dean and S. Crowsley; *Ninewells Hospital, Dundee* (39) — T.H. Pringle and D. Jamieson; *Wessex Cardiothoracic Centre, Southampton* (39) — J.M. Morgan and R. Williams; *Solihull Hospital, Solihull* (39) — K. Priestley and P. Brennan; *Wordsley Hospital, Stourbridge* (35) — E.J. Flint, P. Forsey, and A. Drewnicki; *Good Hope Hospital, Sutton Coldfield* (30) — R.E.A. Smith and J. Tipping; *Derriford Hospital, Plymouth* (29) — C.J. Burrell and I. Lines; *Southern General Hospital, Glasgow* (26) — D. Murdoch and C. Vaughan; *Ipswich Hospital, Ipswich* (24) — R.M. Oliver and C.J. Woollard; *St. Bartholomew's Hospital, London* (24) — A.W. Nathan and J. Sibley; *Papworth Hospital, Cambridge* (23) — M.C. Petch and S. Newell; *Western Infirmary, Glasgow* (22) — J.D. McArthur and S. Starkey; *Nottingham City Hospital, Nottingham* (20) — A.J. Ahsan and J. Riley; *County Hospital, Hereford* (16) — J. Glancy and D. Thomas; *Stobhill Hospital, Glasgow* (14) — K.J. Hogg and Y. Brown; *Stafford General Hospital, Stafford* (12) — P.A. Woodmansey and M. Batthew; *Royal Sussex County Hospital, Brighton* (12) — S. O'Nunain and L. Bennett; *Manor Hospital, Walsall* (11) — A. Cunnington and C. Boden; *New Cross Hospital, Wolverhampton* (10) — J.W. Pidgeon and K. Nicholas; *Glasgow Royal Infirmary, Glasgow* (9) — S.M. Cobbe and C. Armour; *Hairmyres Hospital, East Kilbride* (7) — K. Oldroyd, B. Vallance, and J. Young; *Glan Clywd Hospital, Bodelwyddan* (6) — G.J. Green and N. Waterfield; *Maelor Hospital, Wrexham* (6) — R.P.W. Cowell and M.E. Antony; *The General Infirmary, Leeds* (5) — G.W. Reynolds and A. Nicholls; *Royal Free Hospital, London* (5) — J.G. Coghlan and W. Smith; *St Mary's Hospital, London* (4) — N.S. Peters and J. Varghese; *Sandwell Hospital, West Bromwich* (4) — P.J. Cadigan and A. Ridney; *Wansbeck General Hospital, Ashington* (4) — B. Thwaites and J. Cronin; *Beaumont Hospital, Dublin* (3) — T. Gumbrielle and J. Bedford; *City Hospital, Birmingham* (3) — T. Millane; *Doncaster Royal Infirmary, Doncaster* (3) — G.E. Payne and V. Hayward; *Heartlands Hospital, Birmingham* (1) — P. Ludman, J.M. Beattie, and S. Ramzan: *Trial Steering Committee* — S.M. Cobbe (chair), S.G. Ball, J.M. Bland, M.J. Buxton, and R. Sutton; *Data Monitoring Committee* — P. Sleight (chair), R.J.C. Hall, S.J. Pocock, and P.A. Poole-Wilson; *End Points and Events Committee* — M.C. Petch (chair), M.B. Buchalter, C.J. Burrell, J.C. Cowan, M.D. Gammage, R.T. Johnston, A.N. Sulke, and G.S. Venables; *Data Management Center (Nottingham Clinical Research Group)* — A.M. Skene (director), L. Brown, A. Charlesworth, M. Goulder, J. Sprague, and S. Stead.

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BRIEF REPORT

A Relapsing Inflammatory Syndrome and Active Human Herpesvirus 8 Infection

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SUMMARY

We describe an immunocompetent 61-year-old woman who was negative for human immunodeficiency virus and who had recurrent human herpesvirus 8 (HHV-8) infection associated with a relapsing systemic inflammatory syndrome characterized by fever, lymphadenopathy, splenomegaly, edema, arthrosynovitis, and rash. Kaposi's sarcoma developed 10 months after the initial clinical presentation. A correlation was documented between the recurrent clinical manifestations and the HHV-8 load in plasma and peripheral-blood mononuclear cells. Histologic examination of an enlarged lymph node heavily infected with HHV-8 revealed an atypical lymphoproliferative disorder characterized by paracortical hyperplasia and collapsed primary and secondary follicles.

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Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma (KS)–associated herpesvirus, is a member of the subfamily Gammaherpesvirinae and is etiologically linked to KS,[1] primary effusion lymphoma (body-cavity–based B-cell lymphoma),[2] and the plasmablastic form of multicentric Castleman's disease.[3] Nonspecific signs and symptoms, such as fever, arthralgia, splenomegaly, lymphadenopathy, diarrhea, and fatigue, have been reported both in immunocompromised patients[4,5] and in otherwise healthy subjects[6,7] in association with HHV-8 antibody seroconversion. However, the clinical manifestations of primary or reactivated HHV-8 infection continue to be updated. The levels of HHV-8 DNA in plasma and peripheral-blood mononuclear cells have been associated with the risk of KS[8,9] as well as with the activity and exacerbation of HHV-8–associated multicentric Castleman's disease.[10]

We describe a previously healthy woman who was negative for human immunodeficiency virus (HIV) and who had recurrent episodes of active HHV-8 infection, in whom a relapsing systemic inflammatory syndrome, characterized by fever, lymphadenopathy, edema, arthrosynovitis, and erythematous rash, preceded and accompanied the development of KS. A correlation was observed between the clinical manifestations and the levels of HHV-8 replication.

CASE REPORT

In February 2000, a 61-year-old woman was referred to our hospital with a one-year history of low-grade fever, lymphadenopathy, splenomegaly, arthralgia, edema, and rash involving the face and limbs. Her medical history was unremarkable. In February 1999

she had been hospitalized elsewhere. On that occasion, laboratory studies showed mild anemia, an elevated erythrocyte sedimentation rate (34 mm per hour; normal range, 2 to 15), and elevated levels of C-reactive protein (63 mg per liter; normal range, 2 to 6), lactate dehydrogenase (751 U per liter; normal range, 210 to 425), and beta$_2$-microglobulin (6.6 mg per liter; normal, less than 3.0). To rule out a lymphoproliferative disorder, the patient underwent an axillary–lymph-node biopsy, which was described as showing "nonspecific inflammatory changes," and a bone marrow core biopsy, the results of which were reported to be normal. She was treated with antibiotics, acetaminophen, and nonsteroidal antiinflammatory drugs, with minimal benefit. In November 1999, multiple cutaneous nodules with normal overlying skin, Raynaud's phenomenon, and diffuse severe arthrosynovitis developed. A biopsy of a skin nodule was diagnostic of KS. No specific treatment was instituted.

At the time of admission to our hospital, in March 2000, the patient appeared fatigued but not in acute distress. Her body temperature was 37.5°C. A few lymph nodes, each less than 2 cm in diameter, were palpable in the cervical, axillary, and inguinal regions. Severe arthrosynovitis of the hands, wrists, elbows, and knees was present. A nonpitting edema and a nonpruritic, nonpalpable, erythematous rash that involved the face and limbs and blanched on diascopy, as well as multiple cutaneous nodules with normal overlying skin, were present (Fig. 1). The spleen was palpable. The findings on physical examination were otherwise normal. Routine laboratory examination showed mild anemia and leukopenia with a normal differential count, increased levels of lactate dehydrogenase and C-reactive protein, and an increased erythrocyte sedimentation rate. Tests for rheumatoid factor, antinuclear antibodies, anti-DNA antibodies, and antibodies against extractable nuclear antigens were negative, as were direct and indirect Coombs' tests. The results of the immunologic studies are summarized in Table 1. Findings on examination of a skin-nodule biopsy specimen were diagnostic of hypodermic KS.

Because of the rapid appearance and progression of the skin lesions, the patient was treated with liposomal daunorubicin (40 mg per square meter of body-surface area intravenously) for two months, with improvement of the edema, arthritis, and rash and disappearance of the fever and skin nodules. In June 2000, however, the patient was readmitted for recurrence of fever (temperature, up to 39°C), fatigue, diffuse arthrosynovitis, edema, and rash. Total-body computed tomography showed cervical, axillary, thoracic, and abdominal lymphadenopathy accompanied by marked splenomegaly (longitudinal diameter of the spleen, 17 cm). Examination of an excised axillary lymph node was negative for lymphoma or KS but showed features consistent with the presence of an atypical immunoproliferative disorder. In view of the recurrence of symptoms and the detection of HHV-8 DNA in plasma and lymph-node tissue, antiviral therapy with foscarnet sodium (180 mg per kilogram of body weight, given intravenously) was begun and was followed by an initial improvement in the patient's condition, but it was discontinued because of worsening renal function and fever.

In September 2000, 17 typical KS skin lesions appeared on the patient's left arm; they were associated with recurrence of edema and an erythematous rash on the face and legs, as well as diffuse arthrosynovitis. The patient was again treated with liposomal daunorubicin, with progressive improvement and regression of sarcoma nodules. In February 2001, the patient once again reported arthralgias, leg and face edema, diffuse rash, and the reappearance of multiple KS skin lesions. Her symptoms worsened over a three-week period, and a high-grade fever developed. Given the reappearance of HHV-8 viremia, the patient was treated with cidofovir (5 mg per kilogram, given intravenously every other week for five months), resulting in the disappearance of all clinical signs and symptoms and a concomitant sustained virologic response. The patient had no symptoms for six months after the termination of therapy, at which time a small KS lesion accompanied by local lymphedema appeared on her left ankle. Three months later, inguinal lymphadenopathy developed. A lymph-node biopsy showed localized KS.

METHODS

IMMUNOLOGIC STUDIES

In vitro proliferation assays for recall antigens were performed according to published protocols.[11] The natural killer cell activity was assessed by means of a standard chromium-51–release assay.

SAMPLE PREPARATION AND QUANTIFICATION OF HHV-8 DNA

DNA was extracted from plasma, peripheral-blood mononuclear cells, or tissue samples according to the phenol–chloroform protocol. The levels of

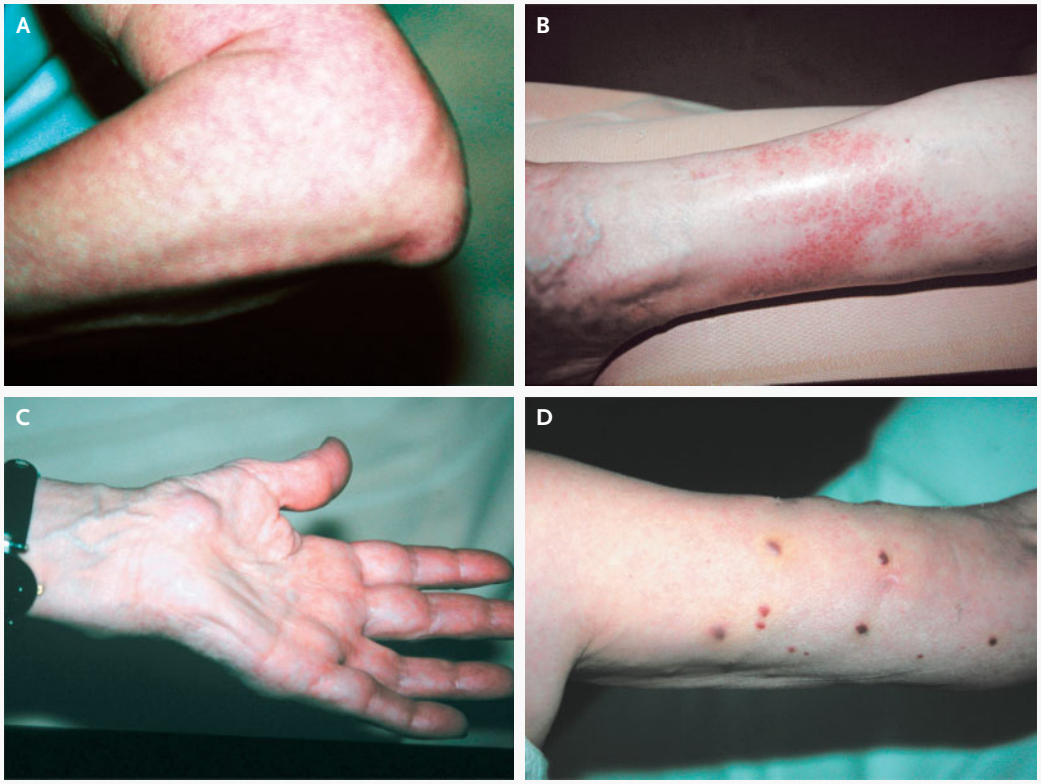


Figure 1. Clinical Signs and Symptoms.
When first admitted to our institution, the patient had a low-grade fever; diffuse lymphadenopathy; nonpitting edema; severe arthrosynovitis of the hands, wrists, knees, and elbows (Panel A); and a nonpruritic, erythematous rash involving the face and limbs (Panel B). Four months before admission, the patient had had multiple cutaneous nodules with normal overlying skin on her trunk, legs, and left hand (Panel C); seven months later, 17 typical KS nodules appeared on her left arm (Panel D).

HHV-8 DNA were determined by a quantitative, calibrated, real-time polymerase-chain-reaction (PCR) assay, as described previously.[12] This assay is based on the use of a specific synthetic calibrator molecule, which allows one to adjust for the difference in the recovery of nucleic acids during extraction and to identify PCR artifacts. The limit of detection was 10 genome equivalents of HHV-8 DNA per milliliter of plasma.

SEROLOGIC ASSAYS

To detect antibodies against lytic and latent antigens of HHV-8, we used immunofluorescence assays based on the HHV-8–positive BCBL-1 cell line and the BCP-1 cell line, respectively, as described previously.[7,13,14]

HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

We used sequential sections of formalin-fixed, paraffin-embedded lymph-node tissue that were 3 μm thick. Monoclonal antibodies or antiserum against CD3 and CD21 (Ylem), CD20, and κ and λ immunoglobulin chains (Dako) and antibody against HHV-8 interleukin-6 (Advanced Biotechnologies) were used according to the manufacturers' instructions or as described previously.[15] The antibody against HHV-8 interleukin-6 specifically recognizes viral interleukin-6 and does not cross-react with human interleukin-6. Paraffin-embedded sections of the lymph node were also tested for Epstein–Barr virus (EBV) by means of in situ hybridization with a fluorescein isothiocyanate–labeled probe specific for EBER-1, an EBV-encoded RNA tran-

Table 1. Immunologic Characteristics of the Patient on First Admission.

Characteristic	Patient's Value	Normal Value
Serum IgG (g/liter)	17.0	8.40–16.6
Serum IgM (g/liter)	1.09	0.48–2.20
Serum IgA (g/liter)	1.01	0.90–3.95
CD4+ T lymphocytes		
% of mononuclear cells	64	32–60
Cells/mm³	840	
CD8+ T lymphocytes		
% of mononuclear cells	23	16–40
Cells/mm³	301	
B lymphocytes (CD19+) (% of mononuclear cells)	4	3–17
Natural killer cells (CD16+) (% of mononuclear cells)	4	4–22
CD4+:CD8+ ratio	2.7	1.3–2.5
Tests		
In vitro cell proliferation (stimulation index)		
Cytomegalovirus antigens	4.5	Reactive if >3.0
Candida antigens	4.9	Reactive if >3.0
Tuberculin purified protein derivative	10.9	Reactive if >3.0
Natural killer cell activity, measured by specific lysis of K562 target cells	Similar to values for 3 age- and sex-matched controls tested in parallel	
Delayed hypersensitivity*	Not anergic	
HIV-1 and HIV-2†	Negative	
Human T-lymphotropic viruses 1 and 2†	Negative	

* A cell-mediated immunity multitest (Pasteur Merieux) was used.
† Enzyme-linked immunosorbent assays, Western blotting, and the polymerase chain reaction were used. The tests were performed at admission, month 7, and month 12.

script (Dako). Sections from normal lymph nodes with mild plasmacytosis were obtained from two age-matched patients and used as negative controls.

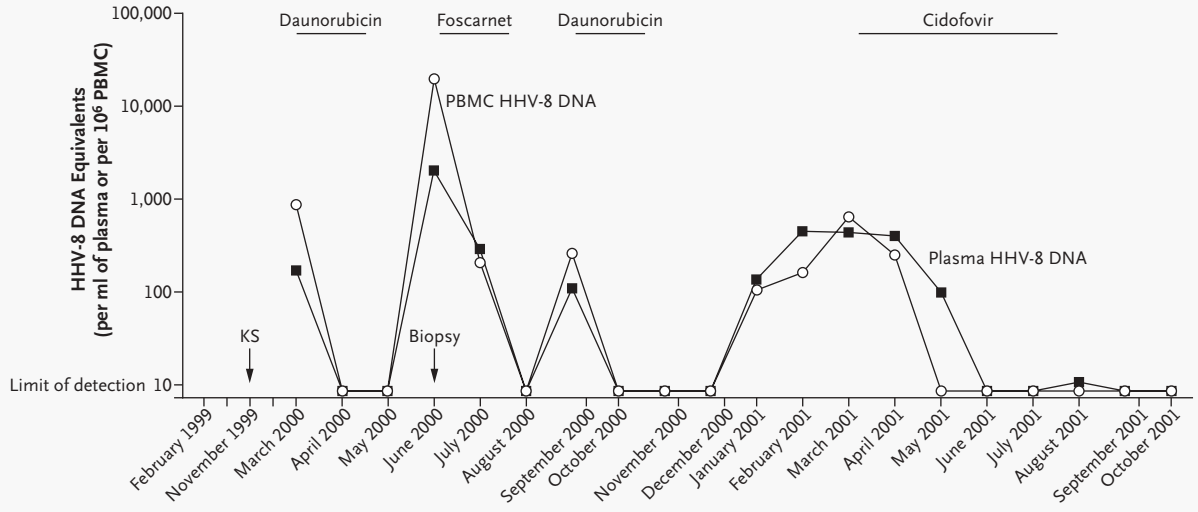
MEASUREMENT OF HUMAN INTERLEUKIN-6 AND INTERLEUKIN-10 LEVELS

Levels of human interleukin-6 and interleukin-10 were measured in sequential plasma samples by means of an enzyme-linked immunosorbent assay (ELISA) (R&D Systems), according to the manufacturer's instructions. Plasma levels of human interleukin-6 and interleukin-10 were considered normal if they were lower than 10 pg per milliliter and 8 pg per milliliter, respectively.[16]

RESULTS

VIROLOGIC AND SEROLOGIC FINDINGS

At the time of the first admission to our institution, the patient had a high viral load (4.2×10^7 genome equivalents of HHV-8 DNA per 10^6 cells) in the excised KS lesion; the levels were lower in peripheral-blood mononuclear cells and plasma (850 genome equivalents per 10^6 cells and 164 genome equivalents per milliliter of plasma, respectively). Treatment with liposomal daunorubicin was followed by clinical improvement associated with rapid abatement of the viremia (Fig. 2). In June 2000, the patient had a recurrence of viremia and a clinical relapse, accompanied by massive and diffuse lymphadenopathy; PCR analysis revealed a very high HHV-8 DNA load in an enlarged lymph node (3.7×10^6 genome equivalents per 10^6 cells). The patient was treated for a short time with foscarnet sodium monotherapy, which resulted in a virologic and clinical improvement. During follow-up, the patient had two more peaks of viremia associated with clinical relapse. In March 2001, cidofovir monotherapy was begun; it resulted in clinical and virologic remission (Fig. 2). Eventually, before the patient was lost to follow-up, reactivation of plasma viremia was



Variable	Value or Finding																					
Temperature (°C)	37.5	38.0	37.5	37.0	37.0	39.0	37.0	37.5	37.5	36.5	37.0	37.0	37.5	38.5	37.5	37.0	36.5	37.0	37.0	37.0	37.0	37.0
Lymphadenopathy	+++	+++	+++	++	++	+++	+++	++	++	++	++	+	+	++	+++	+++	++	++	++	+	+	–
Edema	++	+++	+++	+	+	+++	++	+	++	+	+	–	+	+++	+++	++	+	–	–	–	–	–
Erythematous skin lesions	+	++	+++	++	+	+++	++	+	++	+	+	–	+	+++	+++	+++	++	+	–	–	–	–
Arthrosynovitis	–	+++	+++	++	+	+++	++	+	+++	++	+	–	++	++	+++	++	+	–	–	–	–	–
Longitudinal diameter of spleen (cm)	15		14			17			14					16					12			
Hemoglobin (g/dl)	10.6	10.7	11.5	12.0	12.2	9.0	8.3	10.0	10.0	11.5	11.3	11.8	12.3	8.9	10.2	11.0	11.6	11.2	12.3	11.9	12.5	12.7
White-cell count ($\times 10^{-6}$/mm^3)	6.6	5.5	3.4	3.8	3.6	3.0	2.5	4.6	2.3	3.5	3.3	4.0	4.5	2.5	3.6	2.5	3.8	2.8	3.2	3.0	3.9	4.6
Plasma human interleukin-6 (pg/ml)						70.9		19.4	4.9	1.2	1.9	3.7	6.7	3.9								
Antibodies against HHV-8 (reciprocal of titer)																						
Antibodies against lytic antigens			80		80		1280		320			80			>1280			>1280		160		
Antibodies against latent antigens			640		<40		640		>1280			80			>1280			640		80		

Figure 2. HHV-8 DNA Load, Clinical Signs and Symptoms, and Laboratory Findings during a 20-Month Follow-up.

Sequential blood samples were evaluated to determine the levels of HHV-8 DNA, antibodies against HHV-8 lytic and latent antigens, and human interleukin-6. Clinical signs and symptoms were recorded and graded with the use of a scale ranging from – (absent) to +++ (severe, widespread, or both). Longitudinal analysis revealed a correlation between active HHV-8 infection and the diverse clinical manifestations, whereas human interleukin-6 levels were found to be substantially increased in only two of eight measurements. Therapy consisted of liposomal daunorubicin, foscarnet sodium, and cidofovir. The first KS lesions appeared in November 1999, and an axillary–lymph-node biopsy was performed at our institution in June 2000. PBMC denotes peripheral-blood mononuclear cells.

confirmed, with the reappearance of a KS lesion and, shortly thereafter, the development of KS in a lymph node (64 and 254 genome equivalents per milliliter of plasma, respectively).

As summarized in Figure 2, longitudinal analysis over a period of 20 months demonstrated a remarkable correlation between active HHV-8 infection and the diverse clinical manifestations, including fever, arthrosynovitis, diffuse lymphadenopathy, edema, and rash. Sequential serum samples were positive for IgG antibodies against both lytic and latent HHV-8 antigens (Fig. 2), suggesting that the clinical findings at the time of the first admission to our hospital were unlikely to be due to primary HHV-8 infection. A correlation was seen between increases in both the level of viremia and antibody titers.

No serum sample collected before admission to our institution was available for testing, and therefore we cannot rule out the possibility that the previous symptoms were associated with HHV-8 seroconversion. Extensive immunologic studies showed no evidence of primary or acquired immunodeficiency (Table 1). During follow-up, plasma levels of human interleukin-6 were substantially elevated on only two occasions (Fig. 2), whereas interleukin-10 levels were always within the normal range (data not shown).

HISTOLOGIC FEATURES OF LYMPH NODES

An axillary lymph node removed during the first relapse was moderately enlarged (diameter, 2 cm) and showed substantial effacement of the architecture by a lymphoid infiltrate, which had a predominantly diffuse and focally vaguely nodular pattern of growth (Fig. 3) and comprised small lymphocytes, large lymphoid cells (many with the appearance of immunoblasts), scattered eosinophils, and polyclonal plasma cells, as indicated by immunostaining for immunoglobulin light chains. There was an intense proliferation of postcapillary venules lined by high endothelium. Many of the sinuses were dilated. Lymphoid follicles were difficult to discern, and germinal centers were absent. There was no evidence of necrosis or hyalinized vessels, and mitotic activity was moderate. About 10 percent of plasma cells in the paracortical or interfollicular region were strongly positive for HHV-8 interleukin-6 (Fig. 3D). EBV-encoded RNA transcripts were not detected. No foci of KS were identifiable. Reexamination of the axillary lymph node that had been

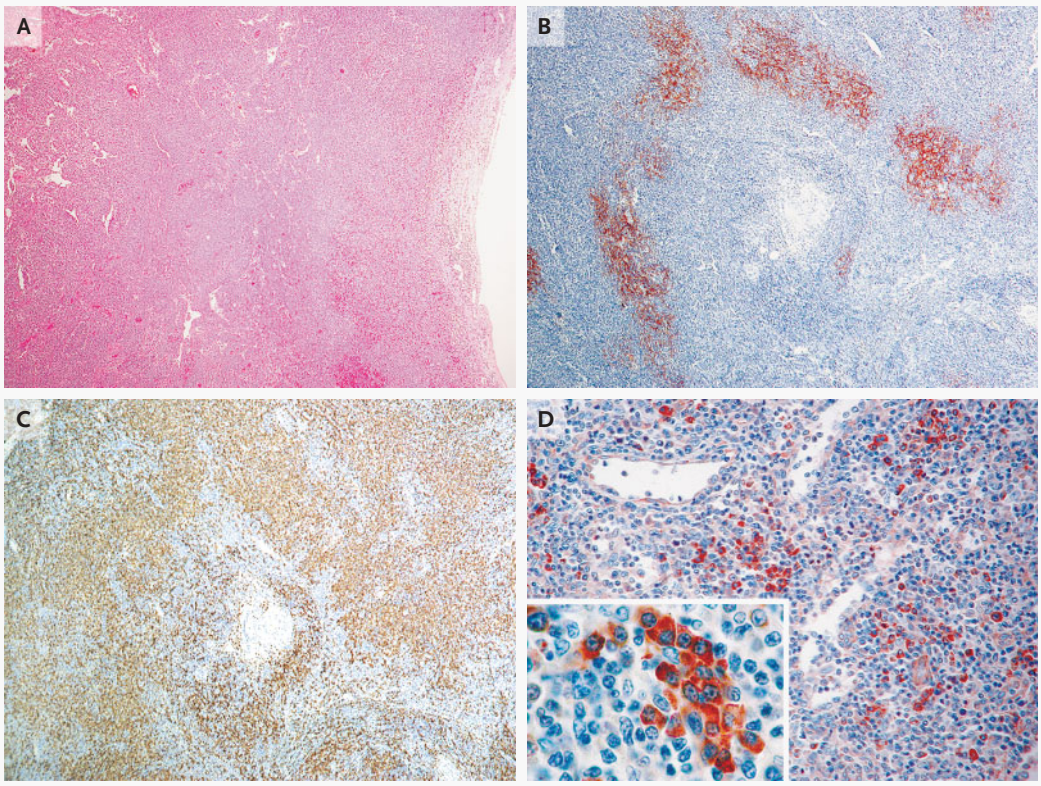


Figure 3. Histopathological Findings in an Enlarged Axillary Lymph Node.

The structure of the lymph node is subverted (Panel A, hematoxylin and eosin): collapsed primary and secondary follicles (Panel B, CD21 reddish-brown immunostaining) are surrounded and infiltrated by T lymphocytes of small to intermediate size (Panel C, CD3 reddish-brown immunostaining); within the expanded paracortex, clusters of plasma cells are productively infected by HHV-8 (Panel D and inset, viral interleukin-6 reddish-brown immunostaining).

excised approximately one year earlier showed similar characteristics, despite the fact that the architecture was more conserved.

DISCUSSION

We describe a relapsing syndrome characterized by fever, lymphadenopathy, splenomegaly, edema, arthrosynovitis, and erythematous rash accompanied by leukopenia and anemia of chronic inflammatory disease in an HIV-negative, immunocompetent woman who had recurrent episodes of HHV-8 reactivation. Over a 20-month period of follow-up, we documented four recurrences of the same signs and symptoms, which closely correlated with the course and extent of HHV-8 replication. In accordance with descriptions of KS and multicentric Castleman's disease in the literature,[9,10,17] exacerbations were always accompanied by spikes of viremia, whereas improvements after therapeutic interventions were consistently associated with an abatement of the viral load. Of particular interest was the finding that antiviral therapy with foscarnet or cidofovir led not only to the suppression of HHV-8 viremia, but also to the concomitant disappearance of both the KS nodules and the other clinical manifestations.

KS is extremely rare in women in the absence of concomitant HIV infection or other causes of immunosuppression. In our patient, however, an extensive search for infection with immunosuppressive retroviruses was unsuccessful, and no clinical or immunologic signs of immune dysfunction were observed.

The extensive lymph-node and splenic involvement suggested the presence of an underlying lymphoma or multicentric Castleman's disease, but both diagnoses were ruled out on the basis of histopathological analysis of two lymph nodes excised at different times. Indeed, the morphologic changes were consistent with the presence of an atypical immunoproliferative disorder characterized by reactive, nonspecific paracortical hyperplasia, which can occur in response to viral infections[18] but to our knowledge has not previously been described in a patient with HHV-8 infection.

As far as Castleman's disease is concerned, the morphologic features of our patient did not satisfy the criteria for either the hyaline vascular or the plasma-cell type of that disorder.[19] Regarding the former, follicles were not prominent, germinal centers were hardly discernible, and the hyaline vascular changes that characterize this entity were absent; regarding the plasma-cell variant, the number of plasma cells present in the excised lymph nodes was limited, and such cells were not arranged in the form of solid sheets. Moreover, we did not detect increased levels of both human interleukin-6 and interleukin-10, as are found during the active phases of HHV-8–associated multicentric Castleman's disease.[10,20-22]

Polyclonal plasma cells in the patient's lymph node were productively infected by HHV-8, as revealed by intense immunostaining for viral interleukin-6.[15,23] This finding, together with the massive viral load in lymph-node–derived mononuclear cells and the presence of splenomegaly and diffuse lymphadenopathy, suggests that secondary lymphoid organs were important reservoirs for viral replication and that viral interleukin-6 may have played a role in some of the systemic manifestations in our patient.

The availability of molecular diagnostic techniques has permitted the association of HHV-8 infection with diseases or syndromes the causes of which were previously unknown.[2-6,24,25] Although HHV-8 is apparently less prevalent in the general population than are other human herpesviruses, the assessment of serum antibody titers and even of cell-associated viral DNA may not provide clinically significant information, since this approach does not distinguish between latent and active infection. Only the integration of these data with measurement of the HHV-8 viral load in plasma and biologic fluids by means of reliable quantitative methods allows the accurate diagnosis of an active infection and thus makes it possible to establish an etiologic link.

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We are indebted to Drs. Juan Rosai and Massimo Freschi for their comments and critical review of the histologic findings, to Drs. Paolo Monini and Barbara Ensoli for their help in setting up the procedures for HHV-8 immunostaining, and to Sara Silva for performing the cytokine assays.

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CLINICAL PRACTICE

Screening for Osteoporosis

Lawrence G. Raisz, M.D.

This Journal *feature begins with a case vignette highlighting a common clinical problem. Evidence supporting various strategies is then presented, followed by a review of formal guidelines, when they exist. The article ends with the author's clinical recommendations.*

At her annual visit, a 60-year-old woman asks her physician whether she should have a bone-density test to screen for osteoporosis. The patient went through menopause at age 52 and received postmenopausal hormone therapy for four years. She takes 500 mg of calcium twice daily and exercises regularly. She has no personal history of fractures, but her mother had a hip fracture at the age of 82. Her height is 63 in. and her weight is 120 lb. What should her physician advise?

THE CLINICAL PROBLEM

Fractures due to osteoporosis are a principal cause of disability and death.[1,2] Approximately 1.5 million fragility fractures (fractures occurring after trauma no greater than a fall from a standing height) occur annually in the United States, and this number will increase as the "baby boomers" reach their 70s. Fewer than one third of patients who have had fragility fractures are appropriately evaluated and treated for osteoporosis,[3-5] despite a high risk of future fractures. The rates of diagnosis are even lower among those who have not yet had a fracture. Practitioners should routinely recommend that patients have an adequate total intake of calcium (1200 mg per day for postmenopausal women) and of vitamin D (400 to 800 IU per day) and participate in weight-bearing exercise — interventions that are safe and inexpensive. However, the incidence of fractures among patients in high-risk control groups who have received calcium and vitamin D in clinical trials is still high.[6] A discussion of pharmacotherapy is beyond the scope of this review, but certain drugs can substantially reduce the risk of fracture in women at high risk for osteoporosis on the basis of bone mineral density and other factors.[7,8] Thus, it is important to identify these patients by appropriate screening.

Current data indicate that too few bone-mineral-density measurements are obtained among patients in high-risk groups.[3] On the other hand, there is a clinical impression that there may be too many measurements obtained among early postmenopausal or premenopausal women, who are at low risk for fracture.

STRATEGIES AND EVIDENCE

Measurement of bone mineral density at the lumbar spine and proximal femur by dual-energy x-ray absorptiometry is a reliable and safe way to assess the risk of fracture in postmenopausal women.[9,10] However, many other factors (which are reviewed below) influence fracture risk and should be considered in making recommendations regarding bone densitometry and therapy.[11,12]

Screening for osteoporosis should ideally provide an estimate of the absolute risk of any fragility fracture during the subsequent 5 or 10 years.[5] More work is needed to refine such predictions, but some estimates are available. For example, the absolute

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10-year risk of a fragility fracture in a postmenopausal woman with a T score indicating low bone mineral density — which is defined as a value 2.5 SD or more below the mean for a young adult (T score, −2.5 or less) — and no other risk factors is less than 5 percent at the age of 50 but more than 20 percent at the age of 65. Absolute risk increases further with additional risk factors, particularly a previous fragility fracture.[13]

Estimates of relative risk asssociated with various factors differ among studies, but there is general consensus regarding the importance of several key factors in risk assessment. In postmenopausal white women, the relative risk of fracture is increased by a factor of 1.5 to 3 for each decrease of 1.0 in the T score, depending on the site measured.[6,9,14,15] The relative risk increases by a factor of 2 to 3 per decade after the age of 50.[16,17] The risk increases by a factor of 1.2 to 2 for patients who have a family history of fracture in a first-degree relative, who weigh less than 126 lb (57 kg), who have recently lost 10 lb or more of weight, who had a delayed menarche (e.g., at an age of more than 15 years), or who currently smoke.[18-21] These factors are also associated with a greater likelihood of low bone mineral density.

The most important risk factor for fracture, independent of bone mineral density, is a previous fragility fracture. This history increases the risk of future fractures by as much as a factor of 8; the risk is highest in the first year or two after the initial episode.[6,22] Silent vertebral fractures (identified radiologically) also increase the risk and should be looked for in patients who have lost more than 2 cm of height.[23] There is also an association between traumatic fractures and osteoporosis,[24] and thus any fracture in a postmenopausal woman should prompt consideration of bone-density measurement.

Falls are another important predictor, particularly for hip fracture in the elderly. Hence, factors that increase the risk of falling — such as impaired vision, neuromuscular deficits, or medications that affect balance — should also be assessed.[25] Several other risk factors should be assessed, although their relationship to bone density and fractures is less clear-cut. Low intake of alcohol (one to two drinks per day) is associated with increased bone mineral density, but higher intakes are associated with low bone mass and an increased risk of fracture, perhaps related to falls.[26] Low 25-hydroxyvitamin D levels (less than 20 ng per milliliter) increase the risk of fragility fractures; this is attributed not only to lower bone mineral density but also to a direct neuromuscular effect of vitamin D that may reduce the frequency of falls.[27,28]

Patients with inflammatory disorders involving the musculoskeletal, gastrointestinal, or pulmonary system and patients who have chronic renal disease or have undergone organ transplantation are also at increased risk for low bone mineral density and fracture.[29,30] Medications, particularly glucocorticoids, may be an aggravating factor. Discontinuation of postmenopausal estrogen therapy may result in accelerated bone loss. Other at-risk populations include patients with hypogonadism due to drugs such as luteinizing hormone–releasing hormone agonists and aromatase inhibitors, with anorexia nervosa, or with the "athletic triad" of low body weight, loss of menses, and low bone mineral density.[31-35] Neurologic diseases can cause bone loss due to immobilization and to the adverse effects of antiepileptic drugs on vitamin D homeostasis.[36,37] Less common causes include congenital abnormalities such as osteogenesis imperfecta and homocystinuria, cancer involving the skeleton (particularly myeloma), and hyperplastic anemia.[30,38]

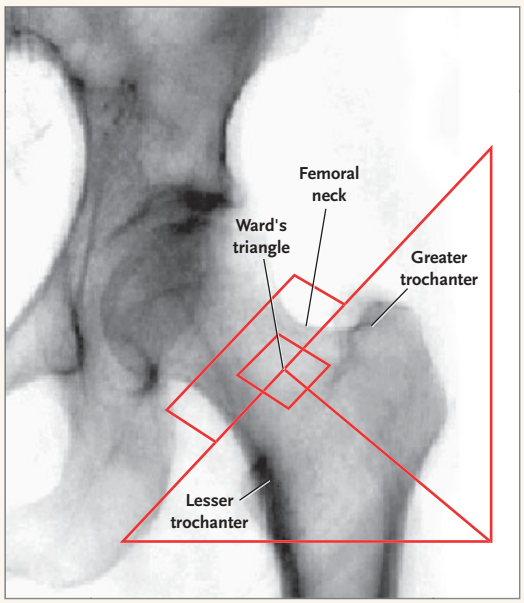
BONE DENSITOMETRY

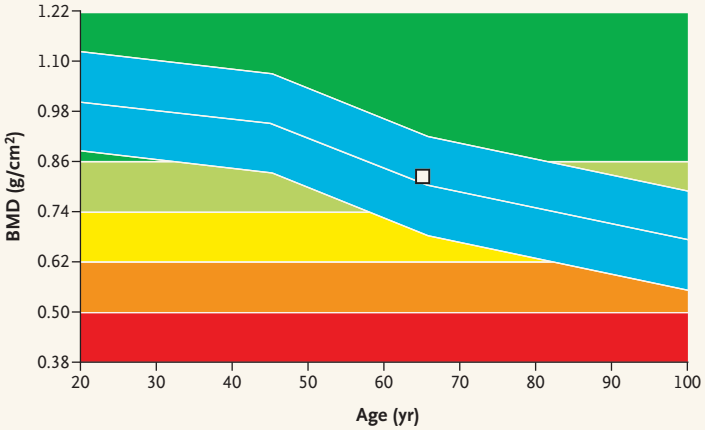
Dual-Energy X-Ray Absorptiometry

In 1991, a consensus panel defined osteoporosis as "a loss of bone mass and microarchitectural deterioration of the skeleton leading to increased risk of fracture."[39] Since microarchitectural deterioration cannot be directly measured, a panel of the World Health Organization (WHO) recommended that the diagnosis of osteoporosis be made when the T score on bone-mineral-density measurement by dual-energy x-ray absorptiometry is −2.5 or lower.[40] They also suggested that the term "osteopenia," or "low bone mass," be applied when T scores are from −1.0 to −2.5. Because there are many more persons with osteopenia than persons with osteoporosis, approximately half of fragility fractures occur in the osteopenic group, although the relative risk of fracture is higher in the osteoporotic population.[14]

The current practice is to perform dual-energy x-ray absorptiometry of the lumbar vertebrae (L1 to L4); the hip, including the femoral neck, Ward's triangle, the greater trochanter, and the total hip (which includes all these measures)[41]; or both (Fig. 1). The results are presented visually, includ-

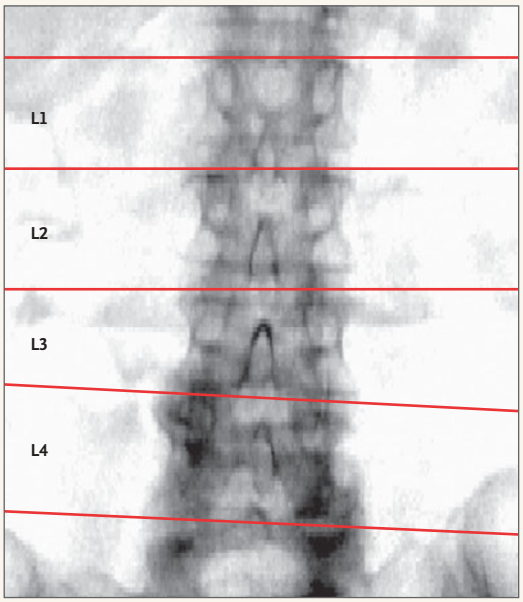
A

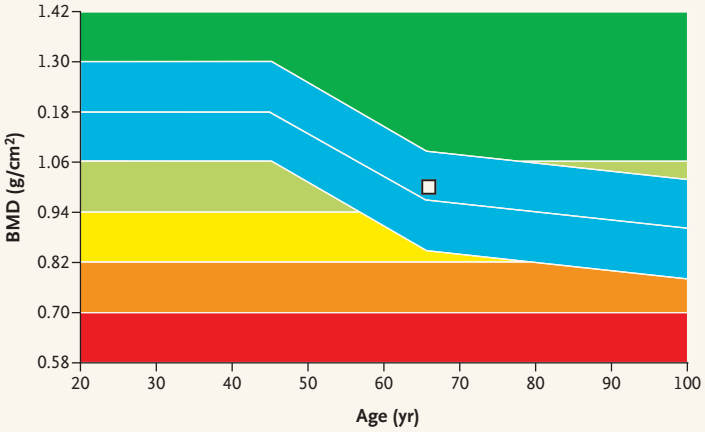




Region	BMD (g/cm2)	Young adult (%)	Young adult (T)	Age-matched (%)	Age-matched (Z)
Neck	0.829	85	–1.3	101	+0.1
Ward's triangle	0.670	74	–1.8	101	+0.1
Trochanter	0.769	97	–0.2	107	+0.5
Total	0.941	94	–0.5	106	+0.4

B





Region	BMD (g/cm2)	Young adult (%)	Young adult (T)	Age-matched (%)	Age-matched (Z)
L1	0.772	68	–3.0	84	–1.3
L2	0.975	81	–1.9	98	–0.2
L3	1.039	87	–1.3	104	+0.4
L4	1.203	100	0.0	121	+1.7
L2–L4	1.086	91	–0.9	109	+0.8

Figure 1. Dual-Energy X-Ray Absorptiometry of the Spine and Hip of a 66-Year-Old Postmenopausal Woman.

A diagnosis of osteoporosis can be made in this patient on the basis of the bone mineral density (BMD) of the hip (Panel A) and the spine (Panel B). The density of lumbar vertebrae 1 through 4 (L1 through L4), both as a percentage of the mean value for a young adult and as a T score, does not indicate osteoporosis, because of the high values for L3 and L4. Since the latter probably reflect osteoarthritic changes in the spine, the use of the two lowest values is recommended for diagnosis.[41] Note that in this patient the T scores at the hip are relatively high, as compared with the spine. The density of the total hip includes the lesser trochanter and adjacent cortical bone and thus has a higher value. The red lines on the left side of each panel indicate the specific area being measured, and the blue areas on the right side indicate the expected age-related means (±1 SD) for white women. Bone-mineral-density measurement results in less than 10 mrem of radiation exposure, as compared with 30 to 60 mrem for a chest radiograph. The color stripes in each panel indicate the degree of concern related to bone density; red denotes high concern and green low concern.

ing both T scores and Z scores (the bone density in the patient as compared with other people of the same age and size expressed as the number of SDs above or below the mean). Of the hip measures, the femoral neck and total hip, in particular, are the most useful in predicting fracture, whereas measurements of Ward's triangle show great variation and are of little clinical value. Although it has been suggested that the WHO definition of osteoporosis should be reserved for patients with low T scores for the total hip, low T scores at other sites are also considered diagnostic of osteoporosis. Spinal measurements may be particularly important in younger postmenopausal women, since they may show osteoporotic values earlier than the hip.

A problem with spinal measurements in older women, however, is that sclerotic changes that occur with age, largely owing to osteoarthritis, may result in an artifactual increase in measured bone mineral density. A careful examination of the actual dual-energy x-ray absorptiometry printout may help resolve this issue. Measurement of mineral density in forearm bone is not used routinely but is recommended for patients with primary hyperparathyroidism, since this site may show the greatest bone loss.[42] Z scores are more informative than T scores in young persons, since the scoring allows comparison of bone density with persons of similar age, height, and weight. More generally, a Z score of −2.0 or lower is considered an indication of the need for more intensive evaluation of possible secondary causes of bone loss,[30,43] although such causes should be considered in all cases.

Quantitative Computed Tomography

Bone density can also be measured by quantitative computed tomography (CT).[44] This technique can analyze trabecular and cortical bone separately and is a sensitive measure of early bone loss in the vertebrae. However, the application of T scores to predict the risk of fracture with the use of quantitative CT has not been validated, and this technique is usually more costly and results in greater exposure to radiation than does dual-energy x-ray absorptiometry.

PERIPHERAL MEASUREMENTS

Because of the limited availability, lack of portability, and relatively high cost of dual-energy x-ray absorptiometry, screening with the use of peripheral densitometry has been developed. These techniques include peripheral dual-energy x-ray absorptiometry, x-ray absorptiometry, and ultrasonography of the radius, heel, and hands. The finding of decreased bone mineral density with these techniques predicts an elevated risk of fracture. However, the interpretation of T scores may not correspond to that of central dual-energy x-ray absorptiometric measurements.[45] Peripheral measurements can be used to assess whether dual-energy x-ray absorptiometry is indicated. To ensure that few patients with low bone mineral density are missed, the use of a conservative cutoff value (for example, a peripheral T score of −1.0 or lower) is prudent. Peripheral measurements should not be used for decision making in regard to diagnosis and management.

SELECTING PATIENTS FOR BONE DENSITOMETRY

A number of recommendations for decision making in regard to bone-mineral-density screening have been developed, but all of these strategies have limitations.[46] The Osteoporosis Self-Assessment Tool, which uses a formula based only on age and body weight, results in a recommendation for testing in 90 percent of women who have osteoporosis but also in as many as 60 percent of women who do not. More complex formulas using other risk factors have not been shown to enhance sensitivity and specificity by very much. Current guidelines (which are summarized below) recommend that all women have a measurement of bone mineral density at the age of 65 years (in selected women, earlier). If these guidelines are used, recommendations in regard to decision making would be needed only for younger women and men, for whom the sensitivity and specificity of the recommendations have not been evaluated.

Strong indicators of the risk of future fracture are considered to be a basis for the recommendation of bone-mineral-density testing before the age of 65 years. A prior fragility fracture warrants bone mineral density testing not only among postmenopausal women but also among men and premenopausal women. A family history of fracture, low body weight, and a loss of either weight (5 percent of baseline weight or more) or height are additional indications,[23,47] as are conditions or drugs known to be associated with bone loss, including primary hyperparathyroidism, hyperthyroidism, hypogonadism (due to disease or drugs), Cushing's syndrome, and long-term glucocorticoid therapy (for example, prednisone at 5 mg or more daily for six months or more[31]). Although there is less evidence on which to base a decision in regard to the

need for bone densitometry in men and in nonwhite postmenopausal and premenopausal women, low bone mineral density also increases the risk of fracture in these groups.[17,48] Hence, pending further information, it is logical to consider a history of fragility fracture, height loss, and the presence of secondary causes of osteoporosis to be indications for bone mineral density measurement.

LABORATORY ASSESSMENT

Laboratory assessment is not used to screen for the presence of osteoporosis but is routinely indicated in patients with low Z scores (for example, −2.0 SDs or below) and may be useful in other patients with low bone density, with the goal of identifying secondary causes (such as elevated serum calcium levels suggesting hyperparathyroidism) or factors that can aggravate bone fragility (such as a low level of 25-hydroxyvitamin D) that can be treated. Clinical or laboratory evidence of disorders such as hyperthyroidism, glucocorticoid excess, gonadal dysfunction, gastrointestinal or renal disease, and cancer warrants appropriate testing. Patients with low bone mineral density and weight loss should be screened for celiac disease, even if they do not have gastrointestinal symptoms.[49]

Biochemical markers of increased bone resorption (collagen cross-links in serum or urine) or increased bone formation (bone-specific alkaline phosphatase and osteocalcin) are associated with an increase in fracture risk.[12] However, these markers show substantial variability, and there are insufficient data to support their use in deciding for or against bone densitometry or pharmacotherapy.[50]

AREAS OF UNCERTAINTY

Data are limited on the relationship between measured bone density and the risk of fracture in premenopausal women, men, and nonwhite persons. There is little information on the effectiveness of screening in enhancing prevention and treatment programs. Data are lacking to guide the frequency of repeated measurements when the initial screening shows normal bone mineral density. Standards are needed for quality control and interpretation of bone-mineral-density tests.[41] Although it is clear that "silent" vertebral compression fractures are associated with an increased risk of fracture,[17] the effect on screening of newer dual-energy x-ray absorptiometry systems, which can measure vertebral-body shape and size and detect such "silent" fractures, is uncertain.[51]

GUIDELINES FROM PROFESSIONAL SOCIETIES

The U.S. Preventive Services Task Force (USPSTF), the National Osteoporosis Foundation (NOF), and the American Association of Clinical Endocrinologists (AACE) have recommended that all women should have a measurement of bone mineral density at the age of 65 years.[52-54] This recommendation is based on the sharp increase in the incidence of fracture that occurs in association with low bone mineral density after the age of 65, as well as clinical trials showing a reduction in the risk of fracture when these women are treated. The USPSTF recommends that women who are 60 to 65 years old and have multiple risk factors undergo bone-mineral-density testing, whereas NOF and AACE suggest that any postmenopausal woman with multiple risk factors should be tested; however, the guidelines do not specify how risk factors should be assessed or weighted. The International Society for Clinical Densitometry and AACE have provided additional guidelines for testing in men, premenopausal women, and children.[41] These guidelines recommend bone-mineral-density testing in patients who have diseases or are receiving drugs that are likely to cause secondary osteoporosis (including glucocorticoids, antiepileptic drugs, luteinizing hormone–releasing hormone agonists, and aromatase inhibitors) and in all patients with fragility fractures.

SUMMARY AND RECOMMENDATIONS

Bone-mineral-density measurements should be obtained routinely in all women over the age of 65 years and in men and younger women who have had a fragility fracture. Compliance with this recommendation alone would be a great advance in comparison with current practice. As is outlined in Figure 2, all patients should be asked about risk factors and secondary causes of osteoporosis and should be advised about the recommended intake of calcium and vitamin D (1200 mg and 400 to 800 IU daily, respectively, for postmenopausal women), weight-bearing physical activity, and the dan-

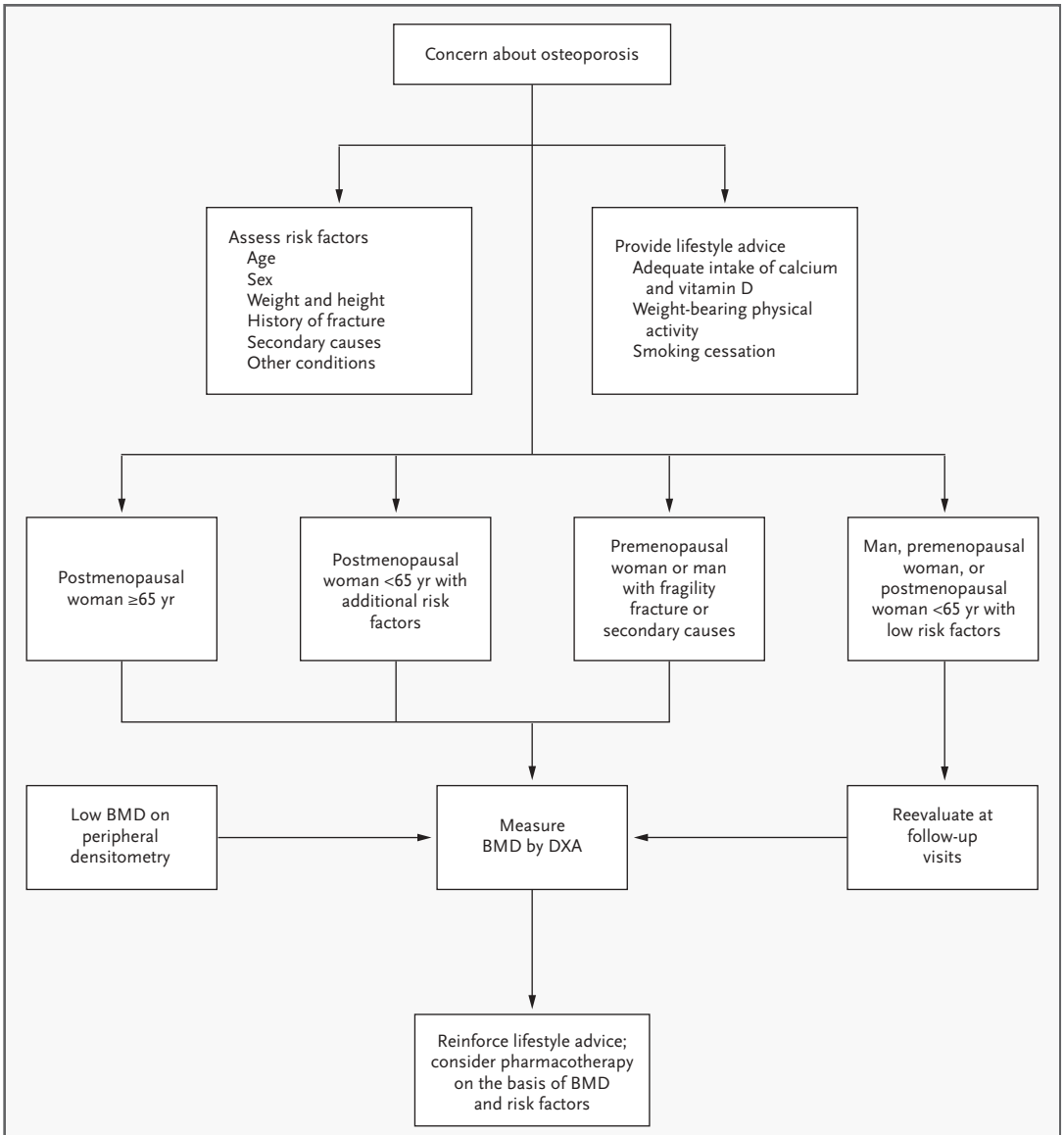


Figure 2. Flow Chart for Recommendations Regarding Selection of Patients for Dual-Energy X-Ray Absorptiometry (DXA).

For peripheral densitometry, each system will have different levels of T-score cutoff. In most cases, dual-energy x-ray absorptiometry will be recommended for patients with T scores of –1.0 or lower. It is important to identify diseases or drugs that are likely to cause skeletal fragility or to increase the risk of falls. Risk factors that routinely warrant bone-mineral-density testing include an age of more than 65 years, a personal history of fracture (particularly fragility fracture) or height loss of more than 2 cm, a family history of fracture in a first-degree relative, low body weight (less than 126 lb), and recent weight loss (more than 5 percent). Other risk factors include female sex, late menarche, early menopause, low calcium intake, vitamin D insufficiency, smoking, excess alcohol intake, physical inactivity and muscle weakness, and impaired vision or balance. Secondary causes of osteoporosis include hyperparathyroidism, hyperthyroidism, Cushing's syndrome, glucocorticoid therapy, inflammatory disorders (including arthritis, bowel disease, and pulmonary disease), hypogonadism (including treatment with luteinizing hormone–releasing hormone agonists and aromatase inhibitors), cancer (especially hematologic conditions), congenital disorders (including osteogenesis imperfecta and homocystinuria), and neurologic disorders (including immobilization and treatment with antiepileptic drugs). BMD denotes bone mineral density.

gers of smoking. The decision to measure bone mineral density in postmenopausal women under the age of 65 should be made on the basis of the presence of risk factors that increase the likelihood of detecting osteoporosis or osteopenia. For example, in the patient described in the vignette, obtaining a dual-energy x-ray absorptiometric scan would be justified on the basis of the patient's family history of fracture, her low weight, and the likelihood that a finding of low bone mineral density would influence her treatment.

Although data to guide the frequency of rescreening are lacking, it would be appropriate to repeat bone mineral density measurement in two years in patients with osteopenia and in three to five years in patients with normal bone density. Many of these patients will not lose bone if they have an adequate intake of calcium and vitamin D and exercise regularly. Risk factors should be reassessed and lifestyle advice reinforced at every visit with the patient. This approach is consistent with the recent Surgeon General's Report on Bone Health and Osteoporosis (www.surgeongeneral.gov).

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REVIEW ARTICLE

MECHANISMS OF DISEASE

Tyrosine Kinases as Targets for Cancer Therapy

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Protein tyrosine kinases (TKs) are enzymes that catalyze the transfer of phosphate from ATP to tyrosine residues in polypeptides. The human genome contains about 90 TK and 43 TK-like genes, the products of which regulate cellular proliferation, survival, differentiation, function, and motility. More than 25 years ago, TKs were implicated as oncogenes in animal tumors induced by retroviruses. However, they were largely ignored in drug development because of a paucity of evidence for a causative role in human cancer and concerns about drug specificity and toxicity. The landscape was changed radically by the success of imatinib mesylate, an inhibitor of the BCR-ABL TK in chronic myeloid leukemia (CML) — a result heralded as a proof-of-principle and a triumph of targeted cancer therapy. TKs are now regarded as excellent targets for cancer chemotherapy, but reality lies somewhere between the extremes of triumph and tribulation. In this article we will review mechanisms of aberrant TK signaling and strategies to inhibit TKs in cancer, summarize the status of TK-directed cancer therapies, and discuss challenges and prospects for the future.

TK Regulation, Dysregulation, and Therapeutic Targeting

Regulation of Normal TK Activity

TKs are divided into two main classes (Fig. 1). Receptor TKs are transmembrane proteins with a ligand-binding extracellular domain and a catalytic intracellular kinase domain, whereas nonreceptor TKs lack transmembrane domains and are found in the cytosol, the nucleus, and the inner surface of the plasma membrane. The enzymatic activities of both types of TK are under tight control, so that nonproliferating cells have very low levels of tyrosyl phosphorylated proteins. The kinase domains of all TKs have a bilobar structure, with an N-terminal lobe that binds ATP and magnesium, a C-terminal lobe containing an activation loop, and a cleft between the lobes to which polypeptide substrates bind.

In the absence of ligand, receptor TKs are unphosphorylated and monomeric, and the conformation of their kinase domains is inactive. In some receptor TKs, the cytoplasmic juxtamembrane region further inhibits the enzyme by interacting with the kinase domain.[2] Receptor TKs become activated when ligand binds to the extracellular domain, resulting in receptor oligomerization, disruption of the autoinhibitory juxtamembrane interaction, and autophosphorylation of a regulatory tyrosine within the activation loop of the kinase (Fig. 1). These changes reorient critical amino acid residues, thereby increasing the catalytic activity of the enzyme. After activation, autophosphorylation generates binding sites for signaling proteins, recruiting them to the membrane and activating multiple signaling pathways.[3]

The nonreceptor TKs, typified by c-ABL, are maintained in an inactive state by cellular inhibitor proteins and lipids and through intramolecular autoinhibition.[1] Nonreceptor TKs are activated by diverse intracellular signals through dissociation of

inhibitors, by recruitment to transmembrane receptors (causing oligomerization and autophosphorylation), and through *trans*-phosphorylation by other kinases (Fig. 1). TK signaling is terminated in part through the action of tyrosine phosphatases that hydrolyze tyrosyl phosphates and by the induction of inhibitory molecules.

MECHANISMS OF TK DYSREGULATION IN CANCER

Given the multiple levels of regulation of TKs, it is not surprising that TKs are dysregulated in cancer cells in several ways (Fig. 2). A common mechanism of TK activation in hematologic cancers is the fusion of a receptor or nonreceptor TK with a partner protein, usually as a consequence of a balanced chromosomal translocation. A frequent feature of the partner protein is a domain that causes constitutive oligomerization of the TK in the absence of ligand binding or physiologic activating signals, thereby promoting autophosphorylation and activation. A primary example of this mechanism is BCR-ABL, the nonreceptor fusion TK in CML, in which a tetramerization domain in BCR overcomes autoinhibition of ABL catalytic activity through oligomerization and autophosphorylation.[4] With some receptor TKs, absence of the juxtamembrane inhibitory domain in the fusion protein contributes to activation.

A second important mechanism of TK dysregulation is a mutation that disrupts autoregulation of the kinase. Mutations in the Fms-like tyrosine kinase 3 (FLT3) receptor in acute myeloid leukemia (AML) render this TK active in the absence of ligand[5]; in another example, small deletions and point mutations in the kinase domain of epidermal growth factor receptor (EGFR) in a subset of non–small-cell lung cancers increase the sensitivity of the receptor to its ligand[6] and alter receptor signaling.[7,8] A third mechanism of TK dysregulation is increased or aberrant expression of a receptor TK, its ligand, or both. Examples include overexpression of the receptor TK ERBB2 (HER-2/neu) in breast cancer and overexpression of a mutant form of platelet-derived growth factor (PDGF), a receptor TK ligand, in dermatofibrosarcoma protuberans with t(11;17). Lastly, increased TK activity can result from a decrease in factors that limit TK activity, such as impaired tyrosine phosphatase activity or decreased expression of TK inhibitor proteins.[9] Aberrant TK activation can increase the survival, proliferation, and cytotoxic drug resistance of malignant cells, and in tumors it can increase angiogenesis, invasiveness, and metastatic potential.

STRATEGIES TO TARGET TKs IN CANCER THERAPY

TKs can be inhibited pharmacologically through multiple mechanisms (Fig. 2). The idea behind much of anti-TK drug discovery is to find small molecules that directly inhibit the catalytic activity of the kinase by interfering with the binding of ATP or substrates. Other anti-TK drugs may inhibit activation of fusion TKs by blocking their dimerization. Antibodies against receptor TKs or their ligands interrupt TK signaling through neutralization of ligand, blockade of ligand binding, receptor internalization, and perhaps antibody-mediated cytotoxicity. The stability of some TKs is regulated by binding to heat-shock proteins (e.g., heat-shock protein 90 [Hsp90]), and inhibitors of Hsp90 can disrupt the binding of client proteins such as BCR-ABL and HER-2, causing their degradation. An important advantage of TK-directed therapy is that it is possible to perform pharmacodynamic studies that correlate inhibition of the targeted TK in cancer cells with clinical responses to the drug.

TKs AS TARGETS IN THE TREATMENT OF MALIGNANT HEMATOLOGIC DISORDERS

IMATINIB MESYLATE: THE FIRST SUCCESSFUL SMALL-MOLECULE TK INHIBITOR

The prime example of a dysregulated TK in the hematologic cancers (Table 1) is BCR-ABL, which has been implicated as the direct cause of CML.[41] Imatinib mesylate (Gleevec), a 2-phenylaminopyrimidine compound that is a specific inhibitor of several TKs — namely, ABL, ABL-related gene product (ARG), c-KIT, and PDGF receptor (PDGFR) — induces complete hematologic and cytogenetic remissions in most patients with chronic-phase CML[10] but is much less effective in the accelerated and blast-crisis phases of the disease.[11] ABL is also activated by fusion to nucleoporin 214 (NUP214) in 5 percent of T-cell acute lymphoblastic leukemias[15] and to ETV6 (also known as TEL) in rare cases of atypical CML and acute leukemia,[13] both potential targets for imatinib.[14]

Imatinib should also have activity in cancers caused by activated PDGFRs and c-KIT. PDGFRα is activated by cryptic interstitial chromosome 4 deletions that generate a FIP1L1–PDGFRα fusion TK in some patients with hypereosinophilic

syndrome[27] or systemic mastocytosis,[29] whereas PDGFRβ is activated in some patients with chronic myelomonocytic leukemia and balanced translocations that lead to fusion of PDGFRβ with one of several partner proteins[31] (Table 1). Imatinib induces dramatic clinical and molecular responses in both diseases.[32,42] c-KIT is activated by point mutations in many cases of systemic mastocytosis or mast-cell leukemia and less frequently in AML. The most common c-KIT mutation involves D816 in the activation loop of the kinase domain, but this mutant is not inhibited by imatinib.[43] The normal c-KIT receptor is expressed on most AML blasts and may be overexpressed and activated in some patients, but the responses of AML to imatinib are variable and do not correlate well with inhibition of KIT signaling.[44]

FLT3: A MAJOR TK TARGET IN AML

FLT3 is a receptor TK expressed on blasts in most cases of AML, and it is activated by duplications within the juxtamembrane domain[5] in 25 to 30 percent of patients and point mutations[21] at D835 in 5 to 7 percent of patients (Table 1). Several inhibitors of FLT3 kinase activity are in various stages of development.[45] They inhibit growth and induce apoptosis in hematopoietic cell lines expressing activated FLT3 and have therapeutic efficacy in murine models of FLT3-induced leukemia.[46] In preliminary clinical trials, the drugs had few adverse effects and reduced circulating and bone marrow blasts in 20 to 50 percent of patients with relapsed or refractory AML.[22-24] Whether FLT3 inhibitors will benefit patients with AML whose blasts overexpress the normal FLT3 receptor is unclear. Such patients often do not have a response to FLT3 inhibitors, even though an autocrine FLT3 loop promotes the survival and growth of some AML blasts in vitro.[47,48]

OTHER TK TARGETS IN MALIGNANT HEMATOLOGIC DISORDERS

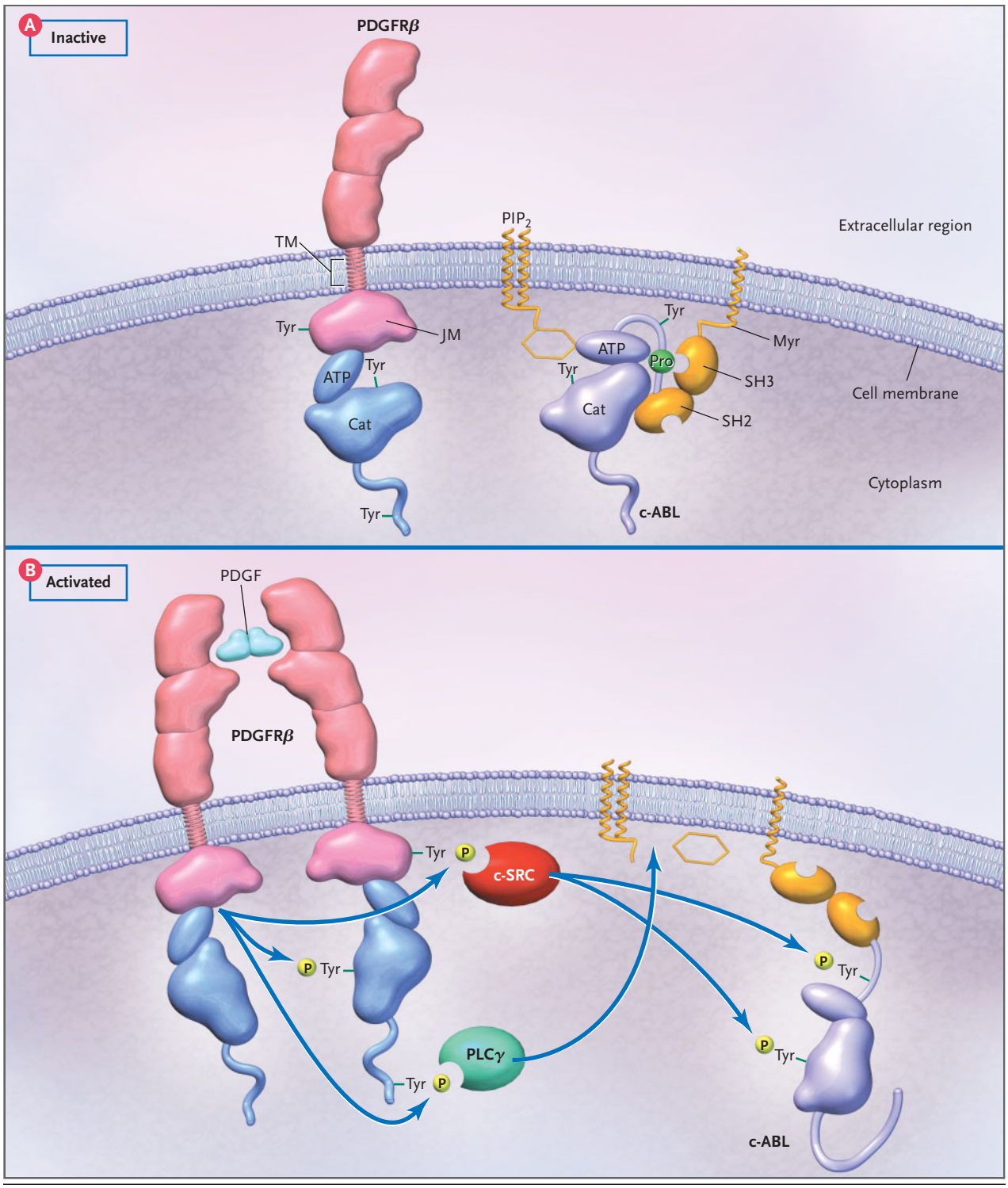
Other TKs have been implicated in hematologic cancers, but targeted therapeutics for these diseases are in their infancy. Fusion of the fibroblast growth factor receptor 1 (FGFR1) TK with one of several partners occurs in the 8p11 myeloproliferative syndrome,[18] and FGFR3 is mutated and overexpressed in multiple myeloma with t(4;14).[20] Translocations involving the anaplastic lymphoma kinase (*ALK*) gene are pathognomonic of anaplastic large-cell lymphoma and generate fusion of the ALK receptor TK with several partners.[16] Fusion of Janus kinase 2 (JAK2), a nonreceptor TK, with TEL or BCR has been described in cases of acute leukemia and atypical CML,[36] whereas an activating point mutation (V617F) in JAK2 is found in the majority of patients with polycythemia vera and in some patients with essential thrombocytosis and idiopathic myelofibrosis.[34,35] Preclinical studies suggest that many of these mutant TKs contribute to the pathogenesis of the disease.

Figure 1 (facing page). Mechanisms of Activation of Normal TKs.

A typical receptor TK (platelet-derived growth factor receptor β [PDFGRβ]) and nonreceptor TK (c-ABL) are depicted, with the ATP-binding (ATP) and catalytic (Cat) lobes of the kinase domains and the transmembrane (TM) region of PDGFRβ indicated. Panel A shows both kinases in their inactive states. Inactive PDGFRβ is monomeric and unphosphorylated, and the catalytic domain is inhibited by protrusion of a regulatory tyrosine (Tyr) in the activation loop into the substrate cleft and by an intramolecular interaction with the juxtamembrane (JM) domain. Inactive c-ABL is associated with the membrane through a covalent N-terminal myristate group (Myr) and is inhibited through intramolecular interaction of the Src homology-3 (SH3) domain with an adjacent proline (Pro) residue and by direct interaction of the catalytic domain with an inhibitory membrane lipid, phosphatidylinositol-4,5-bisphosphate (PIP_2). In Panel B, PDGFRβ is activated upon binding of the ligand (dimeric platelet-derived growth factor [PDGF]), which induces oligomerization of the receptor and intermolecular phosphorylation (P, in yellow) of the activation-loop tyrosine. This leads to a conformational change in the catalytic domain and increased enzymatic activity, while phosphorylation of other tyrosines within the intracellular domain of the receptor creates binding sites for SH2 domain–containing signaling proteins, including c-SRC (red oval) and phospholipase Cγ (PLCγ) (green oval). c-ABL is activated through the phosphorylation of two regulatory tyrosines, one in the activation loop and the other near the SH3 binding site, which can be phosphorylated by another TK, such as c-SRC. In addition, activated PLCγ can hydrolyze and destroy the lipid inhibitor PIP_2. Further detail is provided in the review by Van Etten.[1]

THE DRAMATIC RESPONSE OF CML TO KINASE INHIBITION: THE RULE OR AN EXCEPTION?

The excellent responses of chronic-phase CML and related syndromes to kinase-inhibitor therapy suggest that a dysregulated TK is the sole or predominant somatic genetic abnormality in the malignant cells. In contrast, one or more additional genetic abnormalities are required for the development of AML,[49] which may explain why the activity of FLT3 inhibitors in that disease is only moderate. FLT3 mutations may be acquired late in the



evolution of some cases of AML and therefore in some cases are not central to the origins of the disease.[50] The extreme sensitivity of some cancers to TK inhibition may reflect their absolute dependence on the targeted TK-signaling pathway for survival. This phenomenon of "oncogene addiction"[51] can be seen when imatinib suppresses myeloid colony formation in Philadelphia chromo-

some–positive bone marrow without affecting colonies from normal progenitors.[52]

The responsiveness of some hematologic cancers to kinase-inhibitor therapy may also be due to simultaneous inhibition of more than one TK target. Imatinib inhibits both BCR-ABL and c-KIT in hematopoietic progenitors, and this dual activity may in part account for its efficacy in CML.[53] Similarly, the drug SU5416 inhibits vascular endothelial growth factor receptors (VEGFR) in addition to FLT3 and c-KIT; among patients with c-KIT–positive AML, those who had a response to SU5416 had increased VEGF levels before treatment and decreased bone marrow microvasculature after treatment.[24] The response to this drug may thus be mediated both by inhibition of c-KIT signaling in the blasts and by blockade of VEGFR activation in the bone marrow microenvironment.

TKs AS TARGETS FOR THERAPY OF SOLID TUMORS

IMATINIB TARGETS IN SOLID TUMORS: GIST AND BEYOND

Considerable evidence points to the involvement of TKs in a variety of solid tumors (Table 2). Most gastrointestinal stromal tumors (GISTs) carry mutations in c-KIT that are associated with constitutive activation and receptor phosphorylation.[65,66] GISTs develop at high frequency in adults with germ-line *KIT* mutations, suggesting that c-KIT activation is insufficient for tumorigenesis.[91] Most patients with a GIST who do not have *KIT* mutations have mutations in *PDGFRα*.[85] Trials of imatinib in GIST that were prompted by these findings demonstrated partial responses in more than half the patients[67]; those with *KIT* mutations in exon 11 had the best response, whereas the few patients without either *KIT* or *PDGFRα* mutations did not have a response.[92] In contrast to patients with chronic-phase CML, complete responses to imatinib have not been observed in patients with GISTs,[67] and the varying responses of patients with different c-KIT mutations contrasts with the uniform in vitro sensitivity of these mutant receptors to the drug. Therefore, the dependence of GISTs on c-KIT signaling for survival and proliferation is probably incomplete and complex.

c-KIT and PDGFR have also been implicated in other solid tumors (Table 2), although evidence of the efficacy of kinase inhibitors in these diseases is limited. There are activating kinase mutations in c-KIT in some testicular seminomas[68] and c-KIT mutations or overexpression[69] in small-cell lung cancer, but a phase 2 trial of imatinib in small-cell lung cancer yielded equivocal results.[70] Some glioblastoma cell lines simultaneously express PDGF and its receptors and display evidence of autocrine PDGFR signaling.[81] Imatinib inhibits the growth of these cells[83] and increases their sensitivity to ionizing radiation.[93] Imatinib is also active in dermatofibrosarcoma protuberans,[87] in which platelet-derived growth factor B is fused to collagen type Iα1 and overexpressed.[86] Finally, PDGFRα or PDGFRβ is overexpressed in a subset of sarcomas[72,82,84] and chordomas,[94] some of which respond to imatinib (Table 2).

Figure 2 (facing page). Mechanisms of TK Dysregulation and Therapeutic Targeting in Cancer.

In each case, the TKs known to be activated through that mechanism are listed. Overexpression of a normal receptor TK (here, epidermal growth factor receptor [EGFR]), its ligand, or both is depicted in Panel A. In Panel B, mutations that render a receptor TK constitutively active in the absence of ligand are represented by internal tandem duplications (ITDs) in the juxtamembrane (JM) domain and point mutations (Asp835X) in the activation loop of Fms-like tyrosine kinase 3 (FLT3). In Panel C, BCR-ABL exemplifies the fusion of receptor and nonreceptor TKs to various N-terminal partner proteins as a consequence of chromosomal translocations and deletions. A common feature of the partner proteins is a domain that mediates oligomerization, such as the coiled-coil (CC) domain of BCR. Examples of therapeutic agents targeting TKs are listed in red. Small-molecule TK inhibitors usually act to block binding of ATP or substrate to the catalytic domain of the TK. BCR-ABL may also be targeted by compounds such as 17-allylamino-17-demethoxygeldanamycin (17-AAG) that interfere with binding to cellular chaperones such as Hsp90, by compounds that block oligomerization, by small interfering RNA (siRNA) that induces degradation of *BCR-ABL* mRNA, or by inhibitors of *BCR-ABL* gene transcription. None of these approaches have reached clinical development yet. Finally, receptor TKs and their ligands can be specifically targeted by monoclonal antibodies (top area of Panel A). PDGF denotes platelet-derived growth factor, EGF epidermal growth factor, KL KIT ligand, FL FLIT3 ligand, HGF hepatocyte growth factor, VEGF vascular endothelial growth factor, FGF fibroblast growth factor, PDGFR platelet-derived growth factor receptor, VEGFR vascular endothelial growth factor receptor, FGFR3 fibroblast growth factor receptor 3, ALK anaplastic lymphoma kinase, mRNA messenger RNA, and Ph Philadelphia chromosome.

SMALL-MOLECULE INHIBITORS OF EGFR

EGFR is overexpressed, mutated, or both in many solid tumors (Table 2).[59] Gefitinib (Iressa) and er-

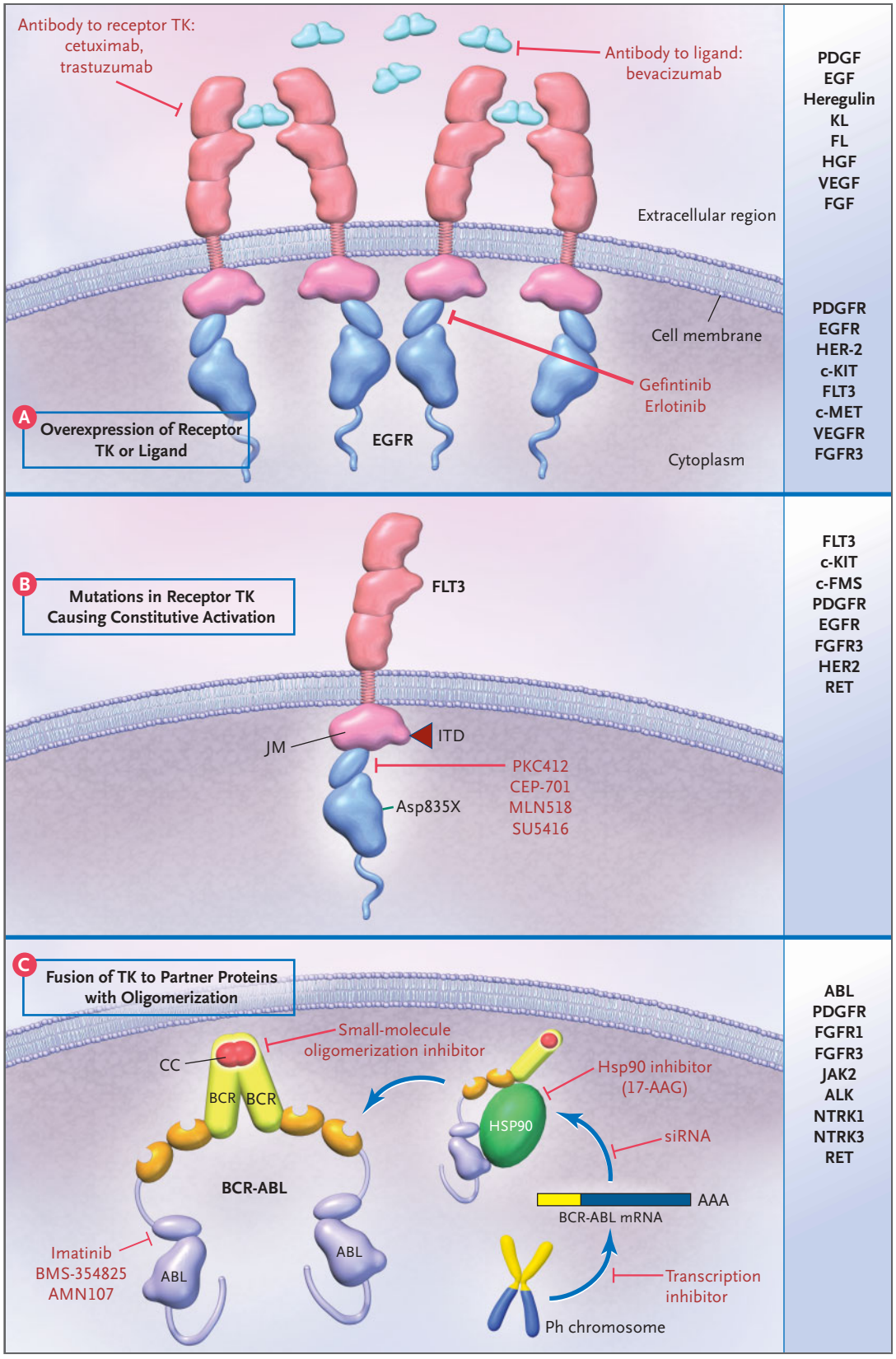


Table 1. Tyrosine Kinase Targets in Malignant Hematologic Disorders.*

Tyrosine Kinase†	Activating Mechanisms	Cancer	Targeted Therapy‡	References
ABL (9q34)				
BCR-ABL	t(9;22)	CML, ALL, AML	Imatinib (not for ALL), SMS-354825,	O'Brien et al.,[10] Druker et al.,[11] Shah et al.[12]
NUP214-ABL	Episomal fusion and amplification	T-ALL	Imatinib§	Graux et al.[15]
ALK (2p23)				
NPM-ALK	t(2;5)	ALCL		Pulford et al.[16]
TPM3-ALK	t(1;2)	ALCL		
ATIC-ALK	inv(2)	ALCL		
CLTC-ALK	t(2;17)	ALCL		
ARG (1q25)				
TEL-ARG	t(1;12)	AML	Imatinib§	Cazzaniga et al.[17]
FGFR1 (8p11)				
ZNF198-FGFR1	t(8;13)	EMS	PKC412, PD0173074§	Macdonald et al.,[18] Chen et al.[19]
FOP-FGFR1	t(6;8)	EMS		
CEP110-FGFR1	t(8;9)	EMS		
HERVK-FGFR1	t(8;19)	EMS		
BCR-FGFR1	t(8;22)	aCML		
FGFR3 (4p16)	Overexpression with t(4;14); K650E	MM	PD0173074,§ SU5402§	Chesi et al.[20]
TEL-FGFR3	t(4;12)	T lymphoma	PD0173074,§ SU5402§	
FLT3 (13q12)	Juxtamembrane internal tandem duplication; D835X; overexpression	AML	PKC412, MLN518, CEP-701, SU5416	Nakao et al.,[5] Yamamoto et al.,[21] Stone et al.,[22] Smith et al.,[23] Fiedler et al.[24]
c-FMS (5q33)	L301F/S; Y969C	MDS, AML		Ridge et al.[25]
NTRK3 (15q25)				
TEL-NTRK3	t(12,15)	AML		Eguchi et al.[26]
PDGFRα (4q12)				
FIP1L1-PDGFRα	Interstitial del(4q12)	HES, SM	Imatinib, PKC412	Cools et al.,[27,28] Pardanani et al.[29]
BCR-PDGFRα	t(4;22)	aCML	Imatinib	Baxter et al.[30]

lotinib (Tarceva) are anilinoquinazolines that are specific, competitive inhibitors of ATP binding by EGFR and were approved by the Food and Drug Administration (FDA) in 2004 for refractory locally advanced or metastatic non–small-cell lung cancer. Gefitinib led to partial responses in 11 to 19 percent of patients with refractory disease in phase 2 trials,[55] whereas erlotinib yielded partial responses in 9 percent of similar patients and improved overall and progression-free survival in a phase 3 trial.[56] Disappointingly, the addition of gefitinib or erlotinib to chemotherapy in the initial treatment of non–small-cell lung cancer did not yield any additional benefit.[95,96]

A small subgroup of patients with non–small-cell lung cancer who frequently had adenocarcinomas and most of whom were Asian, female, and nonsmokers had dramatic and sometimes durable responses to gefitinib monotherapy. Sequencing of the *EGFR* gene in tumor tissue from these patients revealed somatic gain-of-function mutations clustered around the ATP-binding pocket of the kinase domain of the EGFR protein in most cases.[6,7,54] Unlike c-KIT mutations in GIST, these EGFR mutations do not cause constitutive activation; rather, they enhance the responsiveness of the receptor to EGF ligand and increase its sensitivity to inhibition by gefitinib[6,7] and may preferentially activate antiapoptotic signaling pathways in the tumor cells.[8]

Paradoxically, in the Canadian randomized trial of erlotinib in non–small-cell lung cancer, tumor-cell expression of EGFR was significantly corre-

Table 1. (Continued.)

Tyrosine Kinase†	Activating Mechanisms	Cancer	Targeted Therapy‡	References
PDGFRβ (5q33)				
TEL-PDGFRβ	t(5;12)	CMML	Imatinib	Golub et al.,[31] Apperley et al.[32]
HIP1-PDGFRβ	t(5;7)	CMML	Imatinib§	
Rabaptin5-PDGFRβ	t(5;17)	CMML	Imatinib	Magnusson et al.[33]
H4-PDGFRβ	t(5;10)	aCML	Imatinib§	
CEV14-PDGFRβ	t(5;14)	AML	Imatinib§	
JAK2 (9p24)	V617F	Polycythemia vera, essential thrombocythemia openia, idiopathic myelofibrosis		James et al.,[34] Kralovics et al.[35]
TEL-JAK2	t(9;12)	AML, ALL		Lacronique et al.[36]
BCR-JAK2	t(9;22)	aCML		
c-KIT (4q11)	D419X; V560X; D816X; overexpression	AML, SM	Imatinib (not for D816X), SU5416, PKC412	Gari et al.,[37] Furitsu et al.,[38] Zermati et al.[39]
SYK (9q22)				
TEL-SYK	t(9;12)	MDS		Kuno et al.[40]

* CML denotes chronic myeloid leukemia, ALL acute lymphoblastic leukemia, AML acute myeloid leukemia, aCML atypical chronic myeloid leukemia, T-ALL T-cell acute lymphoblastic leukemia, NPM nucleophosmin, ALCL anaplastic large-cell lymphoma, EMS 8p11 myeloproliferative syndrome, MM multiple myeloma, MDS myelodysplastic syndrome, HES hypereosinophilic syndrome, SM systemic mastocytosis, and CMML chronic myelomonocytic leukemia.
† Chromosomal locations are given in parentheses.
‡ Other than imatinib, the drugs listed have not been approved by the Food and Drug Administration.
§ A therapeutic response is predicted on the basis of the identity of the tyrosine kinase but has not been verified clinically.

lated with the response to kinase inhibitor therapy, but *EGFR* mutational status was not.[97] In contrast, studies from Asia, where *EGFR* mutant tumors are more prevalent, have found significant increases in response rates and overall survival among patients with *EGFR*-mutant tumors treated with gefitinib.[98,99] Additional prospective studies are needed to determine whether analysis of *EGFR* expression and mutation in tumors should be used to select patients with non–small-cell lung cancer for EGFR-inhibitor therapy.

EGFR kinase inhibitors are in phase 1 and 2 testing in a wide range of solid tumors.[100] EGFR is overexpressed in about 40 percent of glioblastomas and is activated by extracellular-domain deletions in a subset of these tumors,[57] but gefitinib had only minimal activity in a phase 2 trial in glioblastoma.[58]

TARGETING RECEPTOR TKs AND THEIR LIGANDS WITH MONOCLONAL ANTIBODIES

Receptor TK signaling can also be inhibited by monoclonal antibodies against the receptor or its ligand (Fig. 2). The ERBB2 or HER-2 receptor TK is overexpressed through gene amplification in 20 to 25 percent of invasive primary and metastatic breast cancers and is associated with a poor prognosis.[61] Trastuzumab (Herceptin), a recombinant humanized monoclonal antibody against HER-2, increases response rates and improves survival when added to chemotherapy for metastatic HER-2–overexpressing breast cancer,[63] and in combination with adjuvant chemotherapy, it decreases recurrence in women who have early-stage breast cancer with HER-2 overexpression.[101] Another humanized monoclonal antibody, 2C4, blocks dimerization of HER-2 with other ErbB receptors and is in phase 1 studies.[102] HER-2 is mutated or overexpressed in other cancers,[62] which may also be candidates for anti–HER-2 therapy. A direct inhibitor of HER-2 kinase (CI-1033) is under development.

Several monoclonal antibodies against EGFR are also in development. Cetuximab (Erbitux) is a chimeric antibody against EGFR with activity in combination with chemotherapy in non–small-cell lung cancer, squamous-cell carcinoma of the

Table 2. Tyrosine Kinase Targets in Solid Tumors.*

Tyrosine Kinase†	Activating Mechanisms	Cancer	Targeted Therapy‡	References
ALK (2p23)				
TMP3- or TMP4-ALK	t(1;2)	IMT		Pulford et al.[16]
CLTC-ALK	t (2;17)	IMT		
CARS-ALK	t(2;11;2)	IMT		
ERBB1 (EGFR) (7p12)	G719C/S; deletion $LREA_{747-750}$; L858R; L861Q	NSCLC	Gefitinib, erlotinib	Lynch et al.,[6] Pao et al.,[7] Paez et al.,[54] Kris et al.,[55] Shepherd et al.[56]
	Extracellular-domain deletions vI–vIII	Glioblastoma, NSCLC	Gefitinib, erlotinib§	Frederick et al.,[57] Rich et al.[58]
	Overexpression	SCCHN, NSCLC, ovarian cancer, RCC, pancreatic cancer, colorectal cancer	Gefitinib,§ erlotinib,§ cetuximab, ABX-EGF	Mendelsohn and Baselga,[59] Cunningham et al. [60]
ERBB2 (HER-2) (17q21)	Overexpression; kinase-domain mutations	Breast cancer, lung cancer	Trastuzumab, 2C4, CI-1033	Finn and Slamon,[61] Stephens et al.,[62] Slamon et al.[63]
ERBB3 (12q13)	Overexpression	Soft-tissue clear-cell sarcoma		Schaefer et al.[64]
c-KIT (4q11)	Juxtamemebrane (exon 11) mutations: deletions, V560D/A; extracellular-domain (exon 9) mutations: $dup_{AY502-503}$; kinase domain (exon 13/17) mutations: K642E, D822K/H	GIST	Imatinib	Hirota et al.,[65] Rubin et al.,[66] Demetri et al.[67]
	D816V/H; N822K; Y823D/C	Seminoma	Imatinib§ (not D816X)	Kemmer et al.[68]
	Overexpression; extracellular-domain and juxtamembrane mutations	SCLC	Imatinib§	Boldrini et al.,[69] Johnson et al.[70]
	Overexpression; extracellular-domain and juxtamembrane mutations	Sarcomas	Imatinib§	Smithey et al.,[71] Tamborini et al.[72]
c-MET (7q31)	Overexpression; truncation	Musculoskeletal tumors		Wallenius et al.[73]
	Juxtamembrane mutations: R988C, T1010I	SCLC		Ma et al.[74]
	TPR-MET fusion	Gastric cancer		Soman et al.[75]
	Kinase-domain mutations: M1268T, M1149T	Renal papillary carcinoma		Schmidt et al.[76]
	Overexpression	Malignant melanoma		Cruz et al.[77]

head and neck, and colorectal cancer. In metastatic, EGFR-positive, chemotherapy-refractory colorectal cancer, cetuximab alone had minimal activity, but when combined with irinotecan it had a 22 percent response rate and modestly increased progression-free and overall survival,[60] leading to FDA approval for this indication in 2004. ABX-EGF is a humanized anti-EGFR monoclonal antibody with activity as a single agent in phase 2 trials in metastatic renal-cell and colorectal carcinoma.[103]

Vascular endothelial growth factor (VEGF) is essential for angiogenesis, and either it or its two receptor TKs (VEGFR-1 and VEGFR-2) are overexpressed in many non–small-cell lung cancers and breast, prostate, renal-cell, and colorectal cancers.[104] A pivotal phase 3 study in metastatic co-

Table 2. (Continued.)

Tyrosine Kinase†	Activating Mechanisms	Cancer	Targeted Therapy‡	References
NTRK1 (1q21)				
Tropomyosin–NTRK1	t(1;1)	PTC		Greco et al.[78]
TPR-NTRK1	t(1;1)	PTC		
TFG-NTRK1	t(1;3)	PTC		
NTRK3 (15q25)				
TEL-NTRK3	t(12;15)	Congenital fibrosarcoma, mesoblastic nephroma, secretory breast carcinoma		Knezevich et al.,[79] Tognon et al.[80]
PDGFRα (4q12)	Overexpression	Glioblastoma, osteosarcoma	Imatinib,§ CT52923	Hermanson et al.,[81] McGary et al.,[82] Kilic et al.[83]
	Overexpression	PAIS	Imatinib§	Zhao et al.[84]
	Kinase-domain mutations: D842V/Y, deletion $DIMN_{842-845}$; juxtamembrane mutations: deletion $RVIES_{560-564}$, V561D	GIST	Imatinib§	Heinrich et al.[85]
	t(17;22) and overexpression of COLIα1–PDGFB ligand	DFSP	Imatinib	Simon et al.,[86] McArthur[87]
RET (10q11)	Cysteine point mutations in exons 7 and 8	MEN-2A, FMTC		Santoro et al.[88]
	Kinase-domain mutation: M918T	MEN-2B		
	RET–PTC gene fusions	Radiation-associated PTC		
ROS (6q22)				
FIG-ROS	deletion(6)(q21;q21)	Glioblastoma, astrocytoma		Charest et al.[89]
VEGFR-1 and VEGFR-2	Overexpression of VEGF ligand	NSCLC and breast, prostate, renal, colorectal cancers	Bevacizumab, anti-VEGFR, VEGFR inhibitor	Hurwitz et al.[90]

* IMT denotes inflammatory myofibroblastic tumor, NSCLC non–small-cell lung cancer, SSCHN squamous-cell carcinoma of head and neck, RCC renal-cell carcinoma, GIST gastrointestinal stromal tumor, SCLC small-cell lung cancer, PTC papillary thyroid carcinoma, PAIS pulmonary artery intimal sarcoma, DFSP dermatofibrosarcoma protuberans, COLIα1 collagen type Iα1, MEN-2A and MEN-2B multiple endocrine neoplasia types 2A and 2B, FMTC familial medullary thyroid carcinoma, and VEGF vascular endothelial growth factor.
† Chromosomal locations are given in parentheses.
‡ Gefitinib, erlotinib, cetuximab, trastuzumab, imatinib, and bevacizumab have been approved by the Food and Drug Administration; the other drugs listed have not been approved.
§ A therapeutic response is predicted on the basis of the identity of the tyrosine kinase but has not been verified clinically.

lorectal cancer demonstrated that the addition of bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody, to irinotecan, fluorouracil, and leucovorin led to significant prolongation of survival.[90]

OTHER TK TARGETS IN SOLID TUMORS

Several other TKs have been implicated in solid tumors, but targeted therapies against them are not yet available (Table 2). Activating point mutations and fusions of the receptor TK RET occur in multiple endocrine neoplasia and radiation-associated thyroid cancer.[88] Similarly, the receptor TK MET is overexpressed in melanoma and musculoskeletal tumors, activated by point mutations in small-cell lung cancer and renal papillary carcinoma, and dysregulated by fusion with TPR in gastric carcinoma. The receptor TKs ROS, ALK, NTRK1,

and NTRK3 are activated through the generation of fusion proteins in a diverse set of carcinomas and sarcomas. ALK and NTRK3 fusion proteins are also found in hematologic cancers. The type of disease is dictated by the tissue of expression; for example, TEL-NTRK3 fusions found in congenital fibrosarcoma can induce hematologic cancer when expressed directly in bone marrow.[105]

CURRENT CHALLENGES AND FUTURE DIRECTIONS

LIMITATIONS OF TK-TARGETED THERAPIES: TOXICITY AND RESISTANCE

Targeted cancer therapies should be less toxic than conventional chemotherapy because they are specific for tumor cells. Consistent with this expectation, a maximum tolerated dose for imatinib was never reached in the phase 1 trials of this agent. However, some toxic effects of TK-targeted therapies may be related to inhibition of TKs in normal tissues and therefore difficult to eliminate. Defects in cell-mediated immunity have been reported in patients with imatinib-treated CML and may be a consequence of blockade of c-ABL signaling in T lymphocytes.[106] The cardiotoxicity of trastuzumab may reflect a requirement for HER-2 signaling in cardiomyocytes, whereas the acneiform rash frequently seen in patients who have a response to gefitinib and cetuximab may correlate with inhibition of EGFR signaling in skin.

Resistance to TK-targeted therapies is a growing problem and may be due to any of several mechanisms (Fig. 3). Resistance to TK inhibitors was first identified in patients with advanced CML who had a relapse while receiving imatinib and was associated with point mutations that rendered the ABL kinase resistant to the drug or, less commonly, was associated with *BCR-ABL* gene amplification.[107] A long list of imatinib-resistant BCR-ABL mutants has been linked to drug resistance[108] and, in some cases, to disease progression.[109] Kinase mutations also play a role in acquired resistance to TK-inhibitor therapy in GIST and non–small-cell lung cancer.[110,111] A proportion of the most primitive cancer stem cells or initiating cells, including quiescent Philadelphia chromosome–positive CD34+ cells in CML,[112] may be resistant to TK-targeted therapy — a finding that may account for the relatively low frequency of imatinib-induced molecular remissions in CML.[113] Mechanisms of resistance to monoclonal-antibody therapies targeting receptor TKs are poorly understood, but they are thought to include receptor down-regulation and loss of TK-inhibitory pathways.[114]

Several strategies may prevent or overcome resistance to TK-targeted therapies. In CML, there are several second-generation ABL kinase inhibitors with increased potency and activity against most imatinib-resistant BCR-ABL mutants.[12] Drugs that irreversibly inactivate the kinase[115] or that block substrate binding[116] may overcome "gatekeeper" mutations (such as T315I in ABL), which cause resistance to all ATP-competitive inhibitors. Combinations of monoclonal antibodies against receptor TKs and small-molecule TK inhibitors of the receptor in solid tumors are under investigation. Another approach is to combine a TK inhibitor with cytotoxic chemotherapy or with drugs targeting other signaling pathways in the cancer cell. In colorectal cancer, cetuximab and bevacizumab show benefit only when combined with chemotherapy, but the mechanism is not understood. Finally, immune mechanisms may eradicate residual malignant disease, and trials of TK inhibitors in combination with adoptive immunotherapy or tumor-cell vaccines are warranted.

STRATEGIES TO IDENTIFY NEW TK TARGETS IN CANCER

Constitutive activation of one or more TKs or downstream signaling pathways is likely in many if not most cancers, and we have only begun to identify them. Modern cytogenetics may be able to identify additional TKs activated through chromosomal translocations, rearrangements, and deletions in cancer cells. FISH analysis revealed *ABL1* gene amplification in a subset of T-cell acute lymphoblastic leukemia[117] and led to the identification of the NUP214–ABL fusion protein.[15] In the hypereosinophilic syndrome and glioblastoma, activated fusion proteins involving the PDGFRα and ROS receptor TKs are generated through interstitial chromosomal deletions too small to be detected by routine cytogenetics,[27,89] but they might be revealed through array comparative genomic hybridization. A serendipitous approach, illustrated by the discovery of the FIP1L1–PDGFRα kinase in hypereosinophilic syndrome,[42] is to test each new TK inhibitor in a wide range of patients with cancer and to investigate the identity of the sensitive kinase in any patient who has a response. Flow-cytometric tech-

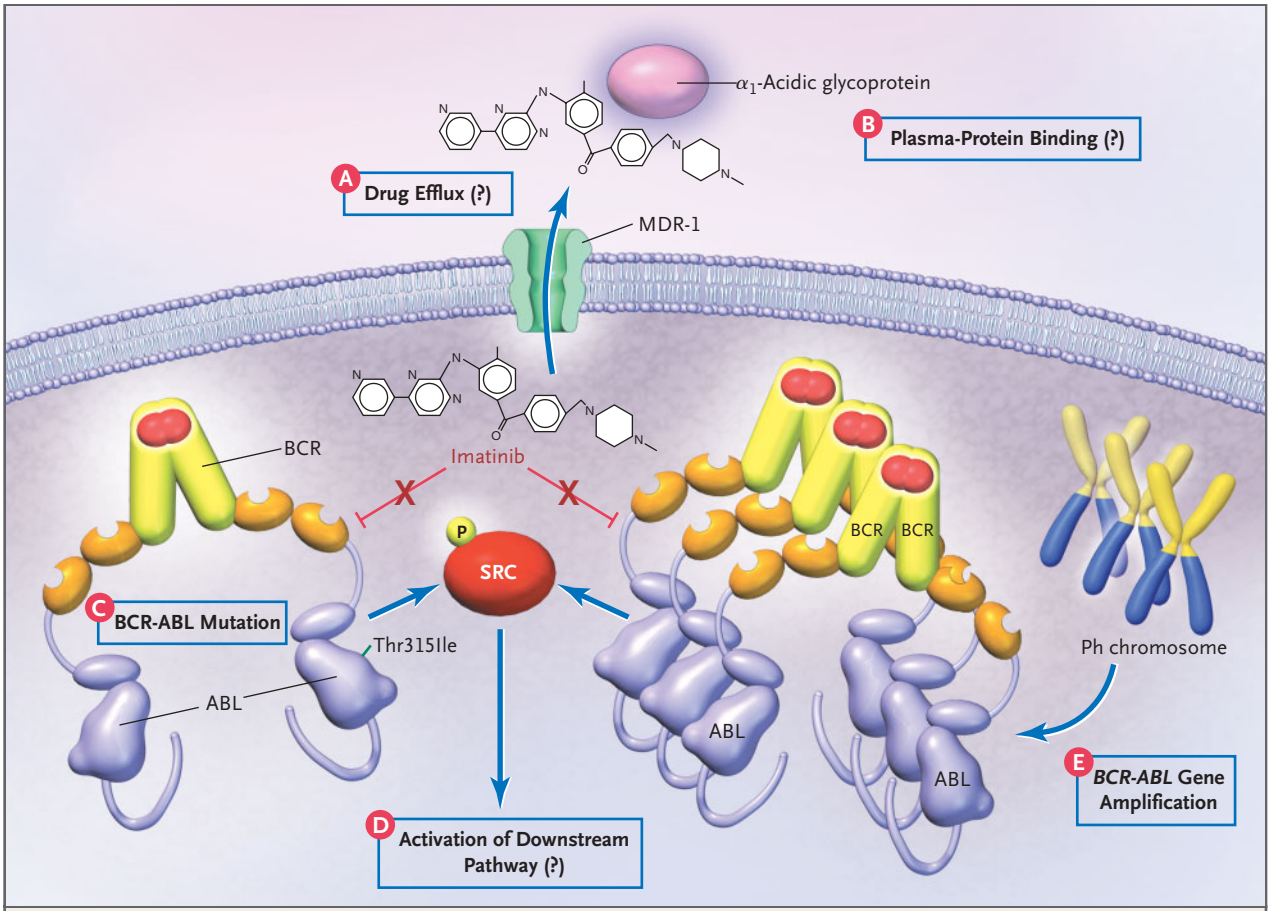


Figure 3. Mechanisms of Resistance to TK-Inhibitor Therapy.

Although causes of imatinib resistance in chronic myeloid leukemia are depicted here, these mechanisms are applicable to both small-molecule TK inhibitors and monoclonal antibodies against receptor TKs. Increased efflux of drug from the cancer cell, mediated by membrane transporters such as multidrug-resistance gene 1 protein (MDR-1), can decrease intracellular concentrations of the drug (mechanism A). Increased drug binding by plasma proteins such as α_1-acidic glycoprotein can decrease the drug's effective concentration (mechanism B). Mutations in the BCR-ABL kinase domain, including Thr315Ile, can decrease or abolish the inhibitory effect of the drug (mechanism C). Constitutive activation of a signaling pathway downstream of a TK, such as a SRC family member, can alleviate the dependence of the cancer on the original TK target (mechanism D). Amplification of the *BCR-ABL* gene leading to overproduction of the TK can confer relative resistance to an inhibitor (mechanism E). For imatinib, mechanisms A, B, and D have not yet been identified in patients with drug-resistant CML.

niques to identify activated TKs and signaling pathways in primary cancer cells are showing promise.[118] Finally, a direct genomic approach can be pursued, where each exon of every TK is amplified by the polymerase chain reaction from tumor tissue and analyzed by DNA sequencing for potential activating mutations. This technique has identified several potential new activating TK mutations in colorectal cancer,[119] but it has a low yield. In cancers other than non–small-cell lung cancer, direct sequencing identified *ERBB2* and *EGFR* mutations in only 3 of 303 primary tumors and none of 203 tumor cell lines.[6,62]

ARE CHANGES IN THE DRUG-DEVELOPMENT PROCESS NEEDED FOR TK-TARGETED THERAPIES?

The future of TK-targeted therapeutics in cancer is promising, but some changes in our approach to the development of these drugs may be indicated. Therapeutic agents targeting TKs do not fit well into traditional phase 1, 2, or 3 drug-development programs because their toxicity profile can be low and because the therapeutic responses may be limited to a small subgroup of patients with a given cancer.[120] In phase 2 or 3 studies, patients should be selected on the basis of evidence of activation of the TK target in their tumors, and such studies

should include pharmacodynamic analysis of target inhibition to avoid discarding a potentially valuable therapeutic agent because of perceived lack of efficacy.[121] The future of gefitinib is in jeopardy after a recent large phase 3 trial involving patients with refractory non–small-cell lung cancer showed that the drug conferred no survival benefit when compared with placebo[122] — a surprising finding, given that the expected frequency of tumors with responsive EGFR mutations was 8 to 10 percent and given that the study was powered to detect such differences.

Patient stratification will lead to smaller potential markets for each newly approved TK-targeted cancer therapy, a situation that may discourage the drug-development process at a very early stage. Clinical development of imatinib was nearly abandoned because the market for a drug for CML was perceived to be too small, but the high prevalence of the disease and the durability of clinical responses have driven annual sales to more than $1 billion. However, the prevalence of the other hematologic cancers caused by activated TKs (Table 1) is far lower, and strategies to encourage the development of therapeutic agents targeting uncommon subtypes of cancer are needed.

Finally, some compromise among the pharmaceutical industry, government, and third-party payers will be necessary with respect to the expense of TK-targeted therapies, each of which is priced to generate annual revenues of $20,000 to $30,000 per patient. Limiting targeted therapies to patients identified by molecular profiling as those who may have a response would help control costs, but ultimately we may not be able to afford a pharmacy of such drugs, which often must be combined with other expensive treatments and which in some patients with cancer may offer only modest survival benefits.

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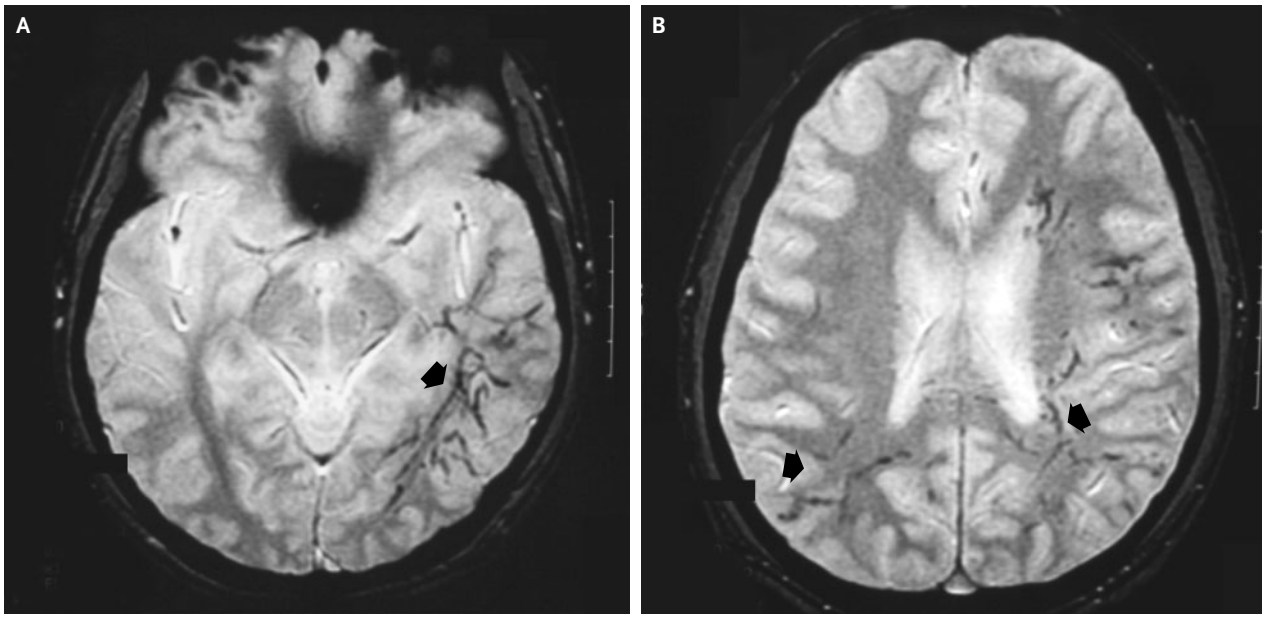
IMAGES IN CLINICAL MEDICINE

Gnathostomiasis — Neuroimaging of Larval Migration



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An 18-year-old man presented with symptoms that evolved over the course of three days and included fever, radicular back pain, vertigo, ataxia, headache, and left-sided hemiparesis. He reported that he had had skin swellings in a migratory pattern during the preceding month. His diet typically consisted of raw fish. A complete blood count showed 7100 white cells per cubic millimeter with 33 percent eosinophils. A computed tomographic scan of the brain showed diffuse swelling. A magnetic resonance image of the brain revealed multiple worm-like lesions in both hemispheres and the cerebellum (arrows, Panels A and B), which did not enhance with the administration of gadolinium. A lumbar puncture revealed an opening pressure of 250 mm of water, the presence of xanthochromia, a white-cell count of 3000 cells per cubic millimeter with 70 percent eosinophils, a protein level of 51 mg per deciliter, and a glucose level of 5.1 mmol per liter. An immunoblot assay of a sample of cerebrospinal fluid was positive for gnathostomiasis. Treatment with albendazole was not given because of concern that severe brain edema might develop if the worms were to die suddenly. Prednisolone was given at a dosage of 60 mg per day for seven days. The patient improved within two weeks, and he was discharged with mild left-sided hemiparesis. At six months after discharge, he had residual mild spasticity of the left leg but no weakness. Gnathostomiasis is typically caused by *Gnathostoma spinigerum* and is most commonly seen in Southeast Asia. The clinical symptoms are related to the mechanical disruption that is associated with the larval migration.

CASE RECORDS of the MASSACHUSETTS GENERAL HOSPITAL

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Case 21-2005: A Four-Week-Old Male Infant with Jaundice and Thrombocytopenia

Nancy C. Andrews, M.D., Ph.D., Sudha Anupindi, M.D., and Kamran Badizadegan, M.D.

PRESENTATION OF CASE

A four-week-old male infant was admitted to this hospital because of jaundice, hyperbilirubinemia, thrombocytopenia, and abdominal distention.

The patient was born at term at another hospital by spontaneous vaginal delivery to a 37-year-old woman (gravida 2, para 2) after an uncomplicated pregnancy. Prenatal screening revealed that the mother had type A Rh-positive blood. Tests for hepatitis B surface antigen and group B streptococcus were negative. The mother was immune to rubella, and a rapid plasma reagin test was nonreactive. Before the delivery, decreased variability was noted on the fetal-heart tracing. At delivery, the infant was limp, cyanotic, apneic, and without a heartbeat. Positive-pressure ventilation administered by bag and face mask was initiated, and chest compressions were performed for approximately two minutes, at which time the infant began crying and the heart rate was greater than 100 beats per minute. He was treated with blow-by oxygen. The infant's Apgar scores, recorded as 0 at 1 minute, improved to 7 at 5 minutes (–1 tone, –1 reflex, –1 color) and to 8 at 10 minutes.

The infant was transferred to a special care nursery in the same hospital, where he was placed in a hood to receive oxygen therapy with a fraction of inspired oxygen (FIO_2) of 0.3. On physical examination, the weight was 3555 g. A systolic ejection murmur was present. The serum levels of electrolytes, calcium, magnesium, and phosphorus were within normal ranges; the results of renal-function tests were normal, and the results of other laboratory tests are shown in Tables 1 and 2. Transient signs of mild respiratory distress, including grunting, developed. Intravenous normal saline (10 ml per kilogram of body weight per day), dextrose (10 percent, 2 ml per kilogram), sodium bicarbonate, erythromycin ophthalmic ointment, vitamin K (1 mg), and hepatitis B vaccine (1 μg) were administered. A specimen of blood was obtained and sent for culture; a chest radiograph showed cardiomegaly. Later that day, the infant was transferred to a tertiary care pediatric hospital. Before the transfer, the oxygen saturation was above 95 percent with the patient in a hood receiving oxygen therapy with an FIO_2 of 0.3, and the respiratory rate was approximately 80 to 85 breaths per minute.

At the second hospital, the temperature was 37°C, the blood pressure 74/46 mm Hg, the heart rate 140 beats per minute, and the respiratory rate 40 breaths per minute. The

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Table 1. Hematologic Laboratory Values.

Variable	First Hospital	Second Hospital					Office Visit	This Hospital
	Day 1 (Birth)	Day 1	Day 2–3	Day 5	Day 7	Day 8	Day 29	Day 30
Hematocrit (%)	37.2	45.1	52.9	56.6	40.5	44.1	32.9	36.1
Hemoglobin (g/dl)	12.9	14.3	16.6	18.7	13.3	14.2	11.3	11.5
Mean corpuscular volume (μm^3)		112.9	110.5	109.1	106.7	105.8	95.2	95
White-cell count (per mm^3)	12,300	12,020	11,210	10,350	5,740	7,740	6,000	7,300
Differential count (%)								
Neutrophils	78	64	66	52			41	45
Band forms	2	6	1	1			1	9
Lymphocytes	18	17	24	25			47	33
Monocytes	2	11	5	15			7	6
Eosinophils	0	2	4	7			4	7
Nucleated red cells (per 100 white cells)	21	29	20	3				
Reticulocytes (%)			9.0		2.5	2.8		
Platelet count (per mm^3)	47,000	38,000	35,000	21,000	37,000	38,000	75,000	67,000
Prothrombin time (sec)					17.6			15.9
Partial-thromboplastin time (sec)					35.7			40.0
Fibrinogen (mg/dl)					118			
D-Dimer (μg/ml)					1.53			

oxygen saturation was 92 percent while the infant was receiving 150 ml of supplemental oxygen per minute. The length was 52 cm (the 90th percentile), the weight 3580 g (75th percentile), and the head circumference 34 cm (approximately the 70th percentile). The infant was a nondysmorphic male in mild respiratory distress with pink skin. Auscultation of the chest disclosed symmetric scattered crackles, and there were mild intercostal retractions. The first and second heart sounds were normal; there was a 3/6 systolic murmur heard best at the clavicular and left sternal border. The precordium was quiet, pulses were 2+ and equal, and the skin was well perfused. The abdomen was soft, the liver extended 2 cm below the right costal margin, and a palpable mass was noted in the left side of the abdomen, which was thought to represent the spleen; bowel sounds were present. The remainder of the examination was normal. The results of laboratory test are shown in Tables 1 and 2.

At the tertiary care hospital, during the first two days of life, phototherapy was administered. Specimens of blood were obtained for culture, and ampicillin and gentamicin were administered. An electrocardiogram revealed right atrial enlargement and right ventricular hypertrophy with a strain pattern and possible biventricular hypertrophy. Chest radiography revealed an enlarged cardiac silhouette and clear lungs. Cardiac ultrasonography showed right ventricular hypertrophy, right ventricular hypertension, mild mitral regurgitation, trace tricuspid regurgitation, septal hypertrophy, a dilated ascending aorta, a patent foramen ovale with bidirectional blood flow, and normal biventricular function. The results were interpreted to be consistent with premature closure of the ductus arteriosus. An abdominal radiograph showed no evidence of bowel obstruction. Abdominal ultrasonography showed mild heterogeneity of the liver, with no evidence of intrahepatic biliary dilatation. The spleen was mildly to moderately enlarged, at approximately 6.9 cm. The kidneys and pancreas appeared to be normal.

On the third and fourth days of life, the patient was jaundiced and hypotonic. A hematologist was consulted; the infant had type A Rh-positive blood, and screening for antibodies to red-cell antigens was negative; a screening for a deficiency of glucose-6-phosphate dehydrogenase, a Coombs' test, and a Heinz-body preparation were all negative. The urinalysis showed trace blood, few red cells, and bilirubin; the results of urine cultures were nega-

Table 2. Chemistry Laboratory Values.*

Variable	First Hospital	Second Hospital							First Hospital		Office Visit	This Hospital	
	Day 1 (Birth)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 8	Day 11	Day 21	Day 29	Day 30	Day 31
Glucose (mg/dl)	22	65			61							81	120
Total bilirubin (mg/dl)		11.6	12	19.0	17.4	21.7	18.2	17.4	16.2	13.0	13.2	15.6	14.6
Direct bilirubin (mg/dl)		2.1	3.6	5.8	8.1	9.3	9.8	9.5	8.7	7.5	7.1	8.3	7.7
Protein (g/dl)											3.3	3.6	3.9
Albumin							1.6	1.7			2.0	2.0	2.0
Globulin											1.3	1.6	1.9
Creatine kinase (U/liter)				215								88	
Creatine kinase MB (ng/ml)				5.1									
Alkaline phosphatase (U/liter)		323		275	324						1241	1507	1404
Aspartate aminotransferase (U/liter)		244		183	155				537	473	383	501	458
Alanine aminotransferase (U/liter)		33		33	35				124	108	64	81	79
Lactate dehydrogenase (U/liter)		2651		1844	1479				1513				
γ-Glutamyl transpeptidase (U/liter)		71		49	48					54			
Ammonia (μg/dl)					103								
Ferritin (ng/ml)												2605	
Thyrotropin (μU/ml)												4.52	
Alpha$_1$-antitrypsin (mg/dl)												128	
Lactate (mmol/liter)					1.0								
Pyruvate (μmol/liter)					0.07								
Pyruvate kinase (U/g of hemoglobin)					15.3								
Immunoglobulin G (mg/dl)						542							
Immunoglobulin M (mg/dl)						20							

* To convert the values for glucose to millimoles per liter, multiply by 0.05551. To convert the values for total and direct bilirubin to micromoles per liter, multiply by 17.1. To convert the value for ammonia to micromoles per liter, multiply by 0.5872.

tive. Oral feedings with formula were begun. Phototherapy was discontinued, and phenobarbital was started at 3 mg per kilogram per day. Blood cultures were negative, and antibiotics were discontinued. A magnetic resonance imaging (MRI) study of the brain revealed patches of mild T_2 hyperintensity in the white matter of the centrum semiovale, which indicates increased water content; there was no sign of restricted diffusion to suggest ischemia. Magnetic resonance spectroscopy showed no abnormalities; there were no signs of hydrocephalus, fluid collections, or masses. The results of a newborn screening showed no abnormalities. One milligram of vitamin K was administered to the infant on the sixth hospital day. Screening tests for galactosemia and tyrosinemia were negative.

On the eighth day of life, the infant was discharged back to the first hospital, still with jaundice and hypotonia. The supplemental oxygen was tapered and then discontinued over the course of the next seven days. The stools were yellow, and there were two brief episodes of arterial-blood desaturation while the infant was feeding. Phenobarbital was discontinued on the 16th day of life. On the 22nd day, the sclerae were icteric, and the jaundice of the skin was resolving. The infant was discharged to his home receiving no medications with plans to return as an outpatient to his pediatrician for further evaluation and follow-up laboratory testing.

Four days later, the father noted that the infant's abdomen was distended. A pediatrician examined the infant three days later and noted abdominal

distention; laboratory tests showed persistent elevation of the bilirubin level, and the liver-function test results were abnormal (Table 2). The infant was admitted to this hospital.

The infant had been feeding well, had good urine output, and had not been febrile. There were no risk factors for sepsis at birth. He lived with his parents and a healthy sibling. The family had no history of liver, gastrointestinal, or congenital heart diseases.

On examination, he was alert and in no distress. The temperature was 37.1°C, the blood pressure 62/42 mm Hg, the heart rate 168 beats per minute, and the respiratory rate 36 breaths per minute. The oxygen saturation was 99 percent while he was breathing ambient air. The weight was 4010 g, the length 54 cm, and the head circumference 36 cm. The sclerae were icteric, and the skin was jaundiced to the lower thighs. A 2/6 systolic ejection murmur was heard over the left sternal border; no gallop or rub was present. The abdomen was soft and moderately distended with active bowel sounds; there was a palpable spleen tip and no hepatomegaly. The remainder of the examination was normal.

The results of laboratory tests on admission are shown in Tables 1 and 2. A radiograph of the chest showed clear lungs and cardiomegaly without pulmonary edema. An abdominal radiograph showed no free air or bowel obstruction. An electrocardiogram showed a normal sinus rhythm, right-axis deviation of +135 degrees, right ventricular hypertrophy, and ST-segment depression in leads V_1 to V_3. Abdominal ultrasonography showed arterialized flow in the left hepatic vein that suggested an arteriovenous fistula, a patent ductus venosus, congested left portal veins, splenomegaly, ascites, and heterogeneous liver parenchyma that was thought to be due to abnormal perfusion. Transthoracic echocardiography with color-flow Doppler revealed mild right ventricular dilation, right ventricular hypertrophy, and prominent portal and hepatic veins. The right ventricular function was normal; there was a left-to-right shunt at the atrial level across a patent foramen ovale and no arteriovenous malformation. The peak pulmonary gradient was 12 mm Hg, the mean pulmonary gradient 5 mm Hg, and the estimated right ventricular systolic pressure 28 mm Hg. The left ventricular function was normal, and the ejection fraction was 65 percent.

The urine was amber colored and clear with a pH of 6.5, specific gravity 1.008, and 2+ bilirubin. Cultures of urine and stool specimens for routine pathogens and cytomegalovirus were negative. A three-day course of vitamin K (4 mg per day) was initiated, and on the second hospital day an oral multivitamin suspension was begun.

On the third hospital day, an MRI of the liver revealed diffuse nodularity that was consistent with cirrhosis. The liver and pancreas were relatively hypointense on T_2-weighted images, except for a small area of hyperintensity in the right lobe of the liver that was thought to represent a cyst. The spleen was moderately enlarged but normal in signal intensity. The left portal vein was more prominent than the right, with numerous possible connections between the left portal vein and the enlarged left hepatic vein. There were some vessels in the superficial aspect of the liver that were thought to be abnormal arteries. The bowel was moderately edematous, the heart had slightly decreased signal intensity, and both kidneys enhanced symmetrically.

A diagnostic procedure was performed.

DIFFERENTIAL DIAGNOSIS

Dr. Nancy C. Andrews: This patient presented at one month of life with signs of liver dysfunction. He had unexplained problems in his first days of life that included right ventricular hypertrophy, thrombocytopenia, and persistent direct hyperbilirubinemia. He did not have lactic acidosis or signs of hemolysis, and the results of tests for galactosemia and tyrosinemia were normal. He was discharged to his home at age three weeks, but he returned to the hospital several days later with abdominal distention, jaundice, and abnormal results on tests of liver function.

May we review the imaging studies?

Dr. Sudha Anupindi: An MRI obtained after the administration of intravenous contrast material revealed a nodular liver with heterogeneous architecture, severe ascites (Fig. 1A and 1B), and decreased signal intensity within the liver and pancreas, but normal signal in the spleen (Fig. 1C). The left lobe of the liver appeared enlarged, and the right lobe was atrophied. This finding is not unusual in chronic liver disease; the caudate lobe of the liver tends to be enlarged in patients with cirrhosis.

A magnetic resonance angiogram showed abnormal vasculature within the liver. The main portal vein was normal in caliber, with an unusually large left portal vein and a hypoplastic right portal

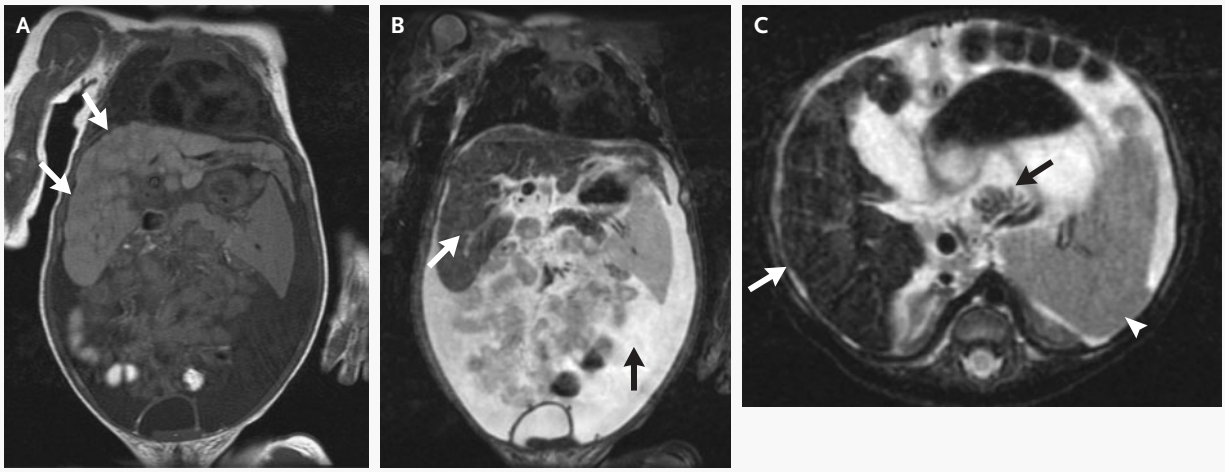

Figure 1. Magnetic Resonance Images of the Liver.

On a coronal T_1-weighted image (Panel A), the liver has a nodular contour (arrows). On a coronal T_2-weighted image (Panel B), the liver has uniformly decreased signal intensity (white arrow). There is a large amount of ascites (black arrow). On an axial T_2-weighted image (Panel C), there is decreased signal intensity within the parenchyma of the pancreas (black arrow); there is markedly decreased signal intensity within the liver (white arrow), but the enlarged spleen maintains normal signal intensity (arrowhead).

vein. Aberrant vessels were also seen in the left lobe. The aberrant vessels and the discrepancy in size between the left lobe and the right are probably a reflection of altered blood-flow patterns.

In summary, the imaging findings were consistent with chronic liver disease and portal hypertension. The decreased signal within the liver and pancreas suggested iron overload.

Dr. Andrews: Iron overload in a newborn is called neonatal hemochromatosis. This rare disorder is prominent in the differential diagnosis of hepatic failure in young infants. There are several causes. Some cases can be attributed to abnormalities in maternofetal iron transfer in the third trimester. In others, tissue iron deposition may be secondary to liver injury.

Neonatal hemochromatosis is typically characterized by fulminant hepatic failure in the first few days of life, with hypoglycemia, coagulopathy, thrombocytopenia, anemia, and renal failure. Circulating alpha-fetoprotein levels, which were not measured in this pregnancy, are usually high, and serum transferrin is usually hypersaturated in an infant who has neonatal hemochromatosis. The ductus venosus is frequently patent, as it was in this patient. Neonatal hemochromatosis is not usually a diagnostic problem. However, the clinical picture can be variable, and patients who present late with the symptoms, as in this case, have been reported.[1]

The MRI scan was helpful in deciding on the diagnosis, because iron deposits were detected in the liver, heart, and pancreas but not in the spleen; these findings are typical of neonatal hemochromatosis. Biochemical abnormalities that are revealed in tests of liver function are relatively nonspecific and could result from other neonatal liver disorders. This patient's elevated serum ferritin level was suggestive but not diagnostic; hepatocyte destruction of any cause leads to the release of intracellular ferritin into the circulation. Also, levels of proteins produced in the liver (e.g., transferrin, ceruloplasmin, and alpha$_1$-antitrypsin) may be depressed in the setting of severe liver damage.

METABOLIC CAUSES OF NEONATAL HEMOCHROMATOSIS

Several metabolic disorders have been reported to be associated with neonatal hemochromatosis (Table 3). This pattern of iron deposition has been seen in infants with defects in bile acid biosynthesis, particularly deficiency of Δ^4-3-oxosteroid 5β-reductase.[3,4] As in this case, infants with a presumed deficiency of Δ^4-3-oxosteroid 5β-reductase have direct hyperbilirubinemia and elevated levels of transaminase but relatively normal levels of γ-glutamyltransferase and normal alkaline phosphatase levels. Infants with these signs tend to present at several weeks of age, although jaundice

Table 3. Disorders Associated with Neonatal Iron Overload.

Disorder	Inheritance	Symptoms or Signs
Neonatal hemochromatosis of uncertain cause[1]	Uncertain; may be autosomal recessive or nongenetic maternal factor[2]	Fulminant hepatic failure in newborn; hypoglycemia, bleeding diathesis, renal failure, nonimmune hydrops due to decreased serum protein; siderosis in liver, exocrine pancreas, and heart with macrophage sparing
Δ^4-3-Oxosteroid 5β-reductase deficiency[3,4]	Autosomal recessive	Controversial; patients with proven mutations in the 5 beta-reductase gene (*SRD5B1*) have not had neonatal hemochromatosis
Maternal Sjögren's syndrome[5]	Nongenetic maternal factor	Congenital lupus syndrome, hepatic insufficiency with hemochromatosis
Trichohepatoenteric syndrome[6]	Uncertain; may be autosomal recessive	Intrauterine growth retardation, trichomalacia, intractable diarrhea, late-onset neonatal hemochromatosis
Hemophagocytic lymphohistiocytosis[7]	Autosomal recessive	Liver failure, elevated serum ferritin, histiocytic infiltration of multiple organs
Maternal infection[8]	Nongenetic maternal factor	Hemochromatotic siderosis, evidence of infection
GRACILE syndrome*[9]	Autosomal recessive	Intrauterine growth retardation, severe lactic acidosis, aminoaciduria, siderosis in liver only

* GRACILE denotes growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death.

is noted at birth. The diagnosis is suggested by the very high levels of 3-oxo-Δ^4 bile acids and allo-(5α-H) bile acids detected in the urine. However, levels of Δ^4-3-oxosteroid 5β-reductase may be decreased when there is liver damage from other causes. Recently, mutations in *SRD5B1*, the gene encoding the reductase, were described in a small series of patients.[10] However, none of the patients were reported to have neonatal hemochromatosis. Thus, the question of whether inherited 5β-reductase deficiency truly causes neonatal hemochromatosis remains open.

Neonatal hemochromatosis has been described as a feature of several complex syndromes. Infants with the trichohepatoenteric syndrome have iron loading in the liver in association with abnormal hair structure and intractable diarrhea.[6] Renal-tubule dysgenesis has also been associated with neonatal hemochromatosis.[11] Pearson's marrow–pancreas syndrome can be associated with hepatic siderosis in the newborn period.[12] However, this infant did not have any of the findings associated with these disorders.

A distinct iron-overload disorder of growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death, termed the GRACILE syndrome, has been described in several Finnish families.[9] It results from a mutation in a gene (*BCS1L*) encoding a mitochondrial chaperone protein that is important for assembly of the respiratory chain. However, patients with the GRACILE syndrome do not have extrahepatic siderosis, as this patient did. Evidence of other defects in the respiratory chain might be sought in this patient, but they are probably ruled out by the absence of a prominent acidosis.

Several forms of anemia, including congenital dyserythropoietic anemias, are associated with iron overload that is due to increased intestinal iron absorption. However, the iron overload associated with these anemias is not apparent until later in childhood, and there was no evidence of a primary erythropoietic defect in this patient.

MATERNAL CONDITIONS ASSOCIATED WITH NEONATAL HEMOCHROMATOSIS

Neonatal hemochromatosis need not be due to a defect in the developing fetus. Although some family pedigrees suggest autosomal recessive inheritance, others include affected half-siblings who have the mother in common but not the father, suggesting the possibility of a maternal factor. This could be a mitochondrial DNA abnormality, but none has been reported to date. Alternatively, it could be an acquired maternal factor, such as an autoantibody. In this regard, neonatal hemochromatosis has been reported in infants of mothers with Sjögren's syndrome who had autoantibodies against Ro/SS-A and La/SS-B antigens.[5] There was no report of maternal autoimmune disease in the case under discussion, though it is still possible that iron loading was the result of an alloimmune process that

took place during gestation. One trial[13] suggested that high-dose intravenous immune globulin therapy improved outcomes in subsequent pregnancies of mothers of infants with neonatal hemochromatosis.

PRIMARY ABNORMALITIES OF IRON HOMEOSTASIS

Mutations in five genes cause iron overload (Table 4). Hemochromatosis in adults is typically a disease of the liver, with less severe manifestations in the heart and pancreas than are seen in infants. In contrast, cardiomyopathy, arrhythmias, and endocrine dysfunction predominate in early-onset hemochromatosis. Classic hemochromatosis is a result of mutations in *HFE.*[14] A similar disease is caused by mutations in transferrin receptor 2 (*TFR2*).[15] The penetrance of *TFR2*-associated hemochromatosis is not known, but hemochromatosis develops in only a fraction of patients who are homozygous for *HFE* mutations. *HFE* mutations have not been identified in patients such as this infant with neonatal hemochromatosis.[2] Most patients with juvenile hemochromatosis are homozygous for mutations in the gene encoding hemojuvelin, a novel protein of unknown function.[16] A subgroup are homozygous for mutations in the gene encoding hepcidin, a peptide hormone that attenuates intestinal iron absorption and cellular iron release.[17] There are no reports of mutations in either of these genes in patients with neonatal hemochromatosis.

In most patients with neonatal hemochromatosis, no cause is identified. The characteristic features include iron deposition in the liver, heart, and exocrine pancreas. The liver shows hepatocellular siderosis and necrosis with giant-cell or pseudoacinar transformation, but no iron accumulation in macrophages of the liver, spleen, or bone marrow. This pattern of iron deposition is similar to that observed in forms of hemochromatosis that have later onset.

The diagnostic procedure performed in this case was probably a biopsy to document tissue iron overload. It could have been a liver biopsy, if blood coagulation could be controlled adequately to make it safe. Alternatively, the biopsy could have been of the oral mucosa to document iron overload in minor salivary glands. This type of biopsy is helpful only if excess iron is detected.

Dr. Nancy Lee Harris (Pathology): Dr. Buie, you

Table 4. Mutated Genes in Hereditary Iron-Overload Disorders.

Gene and Chromosomal Position	Protein	Typical Age at Diagnosis (in Years)	Inheritance	Function of Protein Product	Clinical and Pathological Features
HFE[14] 6p21.3	HFE	40–60	Autosomal recessive (incomplete penetrance)	Atypical major histocompatibility class I protein; interacts with transferrin receptor; function unknown	Liver dysfunction with fibrosis and cirrhosis, cardiomyopathy, endocrinopathies, arthropathy; parenchymal iron overload in liver, heart, and endocrine tissues with sparing of macrophages
TFR2[15] 7q22	Transferrin receptor 2	40–60	Autosomal recessive	Homologue of transferrin receptor; function unknown	Liver dysfunction with fibrosis and cirrhosis, cardiomyopathy, endocrinopathies, arthropathy; parenchymal iron overload in liver, heart, and endocrine tissues with sparing of macrophages
HJV[16] 1q21	Hemojuvelin	15–25	Autosomal recessive	Unknown	Cardiomyopathy and arrhythmias, diabetes, hypogonadotropic hypogonadism, less prominent hepatic dysfunction; tissue iron-loading pattern similar to HFE hemochromatosis but earlier onset, more severe
HAMP[17] 19q13	Hepcidin	15–25	Autosomal recessive	Circulating peptide that attenuates iron absorption, macrophage iron release	Cardiomyopathy and arrhythmias, diabetes, hypogonadotropic hypogonadism, less prominent hepatic dysfunction; tissue iron-loading pattern similar to HFE hemochromatosis but earlier onset, more severe
SLC40A1 2q32	Ferroportin	30–50	Autosomal dominant	Iron transporter that mediates cellular iron release	Hyperferritinemia with or without hepatic fibrosis, cardiomyopathy, diabetes; mild anemia early in course in some patients; macrophage-predominant iron loading

cared for this patient in the hospital; could you give your impressions and tell us about the diagnostic procedure?

Dr. Timothy M. Buie (Pediatric Gastroenterology): Our impression was that this patient had neonatal hemochromatosis. We had test results that ruled out other causes, and the extent of the iron deposition in the organs led us to believe that neonatal hemochromatosis was the most likely diagnosis. We attempted a biopsy of a salivary gland, but we were not able to obtain sufficient tissue and therefore performed a liver biopsy.

CLINICAL DIAGNOSIS

Neonatal hemochromatosis.

DR. NANCY ANDREWS'S DIAGNOSIS

Neonatal hemochromatosis of uncertain cause.

PATHOLOGICAL DISCUSSION

Dr. Kamran Badizadegan: The diagnostic procedure was a liver biopsy, and a core of hepatic parenchyma was obtained. At low magnification (Fig. 2A) the parenchyma shows lobular disarray with prominent bands of fibrosis. The ends of the core are somewhat rounded, which suggests the development of regenerative nodules. Examination after trichrome staining at higher magnification (Fig. 2B) confirms the presence of bands of fibrosis with early regenerative nodules, which are suggestive of progression to early cirrhosis.

A microscopical examination of the hepatocytes shows diffuse damage that includes the loss of normal sinusoidal architecture, pseudogland formation, and giant-cell transformation (Fig. 2C) with focal intrahepatic cholestasis and spotty single-cell necrosis (not shown). There is cytoplasmic pigment in hepatocytes, Kupffer cells, and portal

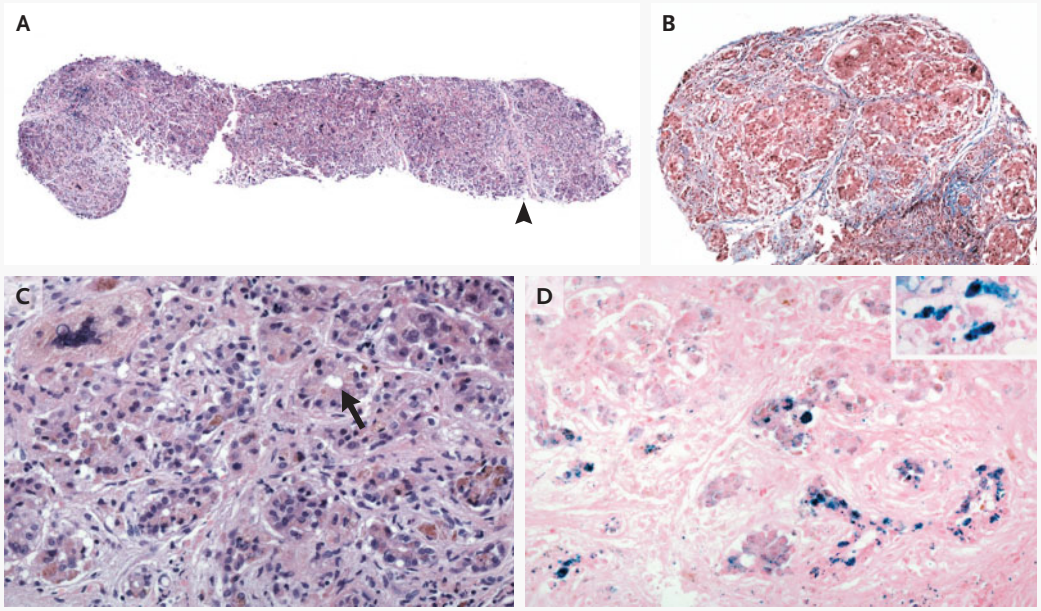

Figure 2. Liver-Biopsy Specimen.

At low magnification (Panel A, hematoxylin and eosin), the specimen from the core biopsy of hepatic parenchyma shows lobular disarray with focally prominent bands of fibrosis (arrowhead). The two ends of the core appear somewhat rounded, suggesting the development of early regenerative nodules. Trichrome staining (Panel B) confirms the presence of bridging and pericellular fibrosis (marked by blue staining) with a focally nodular pattern. A high-power examination of the parenchyma (Panel C, hematoxylin and eosin) shows diffuse hepatocellular damage with pseudogland formation (arrow) and multinucleated giant-cell transformation (top left corner). In addition, there is prominent cytoplasmic pigment deposition seen as brown staining of many of the cells. Prussian blue staining on an adjacent serial section (Panel D) confirms the identity of much of the cytoplasmic pigments as iron. Staining is not limited to the hepatocytes, and reticuloendothelial storage is also present, as seen in three prominent portal-tract macrophages (inset).

macrophages, most of which are positive for the presence of iron, as shown by Prussian blue staining (Fig. 2D). Extramedullary hematopoiesis was prominent. No viral cytopathic changes or microorganisms were identified.

The histologic findings are diagnostic of neonatal hepatitis with giant-cell transformation of hepatocytes. The extent and pattern of iron deposition in the liver biopsy are not specific for disorders of iron metabolism and can be seen with other causes of extensive neonatal liver damage or secondary iron overload. Neonatal hepatitis is a morphologic pattern of injury characterized by diffuse hepatocellular damage, focal hepatocellular necrosis, intrahepatic cholestasis, extramedullary hematopoiesis, and giant-cell transformation of hepatocytes.[18] The diagnosis is typically reserved for infants younger than three months of age in whom extrahepatic biliary obstruction has been ruled out. The morphologic pattern of neonatal hepatitis can be seen in a variety of disorders, including infections and metabolic diseases, many of which are difficult to distinguish on the basis of histopathological findings alone. Although neonatal giant-cell hepatitis is a morphologic manifestation of neonatal hemochromatosis,[19,20] liver biopsy alone is often not sufficient for a definitive diagnosis of this entity in the neonatal period.

Dr. Andrews: There are few treatment options for patients with neonatal hemochromatosis.[21,22] Defects in bile acid biosynthesis, if truly causative in this disorder, may be amenable to oral bile acid therapy. Antioxidant "cocktails" aimed at minimizing oxidative damage catalyzed by free iron appear to be of benefit in some patients. However, in some cases liver transplantation may be the only reasonable option. Counseling for the parents is an important component of management in this disorder, because neonatal hemochromatosis recurs in about 75 percent of subsequent pregnancies.

Dr. Harris: Dr. Buie, would you tell us how you treated this patient and how he is doing?

Dr. Buie: Antioxidant therapy consisting of liquid vitamin E, selenium, and acetylcysteine was administered. The ascites was controlled with diuretics, and we have continued his therapy with multivitamins. The levels of alanine aminotransferase and aspartate aminotransferase are still elevated to about twice the normal values, but clinically he is well. He has no ascites, his height and weight are at the 50th percentile, and he is currently taking no medications. He has persistently elevated levels of alpha-fetoprotein, which in this age group may be associated with inflammation. We are following him closely for signs of hepatocellular carcinoma and portal hypertension.

Dr. Andrews: Not much is known about the natural history of this disorder. It has been reported to resolve in some patients. Recent reports suggest that a specific maternal antibody is involved.[13] If so, the insult would be specific to the fetal period, and this patient would not be likely to have further problems with iron metabolism.

ANATOMICAL DIAGNOSIS

Neonatal giant-cell hepatitis with bridging fibrosis and early regenerative nodules, with iron deposition in hepatocytes and Kupffer cells (consistent with — but not diagnostic of — neonatal hemochromatosis).

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EDITORIALS

Targeting EGFR in Non–Small-Cell Lung Cancer

James H. Doroshow, M.D.

The development of small-molecule inhibitors of the epidermal growth factor receptor (EGFR), such as erlotinib and gefitinib, for the treatment of advanced non–small-cell lung cancer illustrates a pattern of initial overly enthusiastic interest, subsequent critical disappointment, and eventual renewed appreciation of the clinical and mechanistic complexities surrounding the introduction of a new therapy for cancer. Such a pattern has been regrettably common in the history of oncologic therapeutics.

The initial safety and efficacy studies of erlotinib and gefitinib evoked a remarkable degree of optimism that was based on prolonged remissions and, in some cases, dramatic improvements in the quality of life in small numbers of patients whose condition was no longer responding to standard chemotherapy.[1,2] Furthermore, clinical benefit was achieved by treatments that appeared to control, with acceptable levels of toxic effects, advanced non–small-cell lung cancer through a specific molecular mechanism. However, when in randomized clinical trials EGFR inhibitors were combined with cytotoxic chemotherapy, no advantage was demonstrated over standard chemotherapy alone — a result that provoked substantial consternation regarding the potential value of this new class of agents in the treatment of non–small-cell lung cancer and doubts about the clarity of our knowledge of their presumed mechanism of action.[3,4]

In 2004, the public presentation of clinical trial BR.21 conducted by the National Cancer Institute of Canada (NCIC) showing a two-month survival advantage with erlotinib, as is reported in full in this issue of the *Journal*,[5,6] had a positive reception that was particularly warranted because many of the participants had previously received two chemotherapy regimens. As a group, such patients are unlikely to benefit from further cytotoxic approaches. At almost the same moment, interest in EGFR inhibitors was heightened by reports linking mutations in exons 18 through 21 of the *EGFR* gene to the responsiveness of non–small-cell lung cancer to gefitinib.[7,8] Taken together, these findings rekindled substantial enthusiasm for the use of EGFR inhibitors in the treatment of non–small-cell lung cancer. The result has been a flurry of investigative activity to determine the role of these mutations, and potentially that of other alterations in EGFR signaling pathways, in selecting patients who might benefit from EGFR-inhibitor therapy.[9]

The two reports from the NCIC in this issue of the *Journal*, by Shepherd and colleagues[5] on the clinical results and by Tsao and colleagues[6] on the molecular results of a randomized trial comparing erlotinib with placebo in non–small-cell lung cancer, provide data that have a substantial bearing on the treatment of patients with advanced lung cancer. From these studies, critical lessons can also be derived on how anticancer agents that have been selected to affect a specific molecular pathway should be developed.

The NCIC trial is the first randomized study of a tyrosine kinase inhibitor to demonstrate substantial benefit in terms of both relief of symptoms (cough, dyspnea, and pain) and median and one-year survival in non–small-cell lung cancer. This outcome was achieved with mild toxic effects with the use of an oral antineoplastic agent (erlotinib) and led to its approval for use in non–small-cell lung cancer by the Food and Drug Administration in November 2004. Patient characteristics that in phase 2 studies of non–small-cell lung cancer had been associated with responsiveness to EGFR inhibitors — histologic features of adenocarcinoma, female sex, no history of smoking, and Asian an-

cestry — correlated significantly with responsiveness to erlotinib in this trial.

In the accompanying study of potential molecular correlates of sensitivity to erlotinib,[6] paraffin-embedded biopsy and surgical-resection samples of non–small-cell lung cancer, adequate for at least one analysis of EGFR, were available from 328 of the 731 patients enrolled. Response to erlotinib was significantly associated with EGFR positivity on immunohistochemical analysis and increased numbers of copies of the *EGFR* gene. Although twice as many patients with *EGFR* mutations had a response to treatment, as compared with patients whose tumors lacked mutations, this difference was not statistically significant. In the univariate analysis, a survival advantage was associated with increased numbers of gene copies or EGFR expression in patients treated with erlotinib; however, this association was not found in the multivariate analyses.

The importance of these results is heightened by the fact that molecular correlations were performed in the context of a randomized, placebo-controlled study. The observation that the clinical benefit from treatment with erlotinib found in all subgroups that underwent the EGFR analysis was similar to that in the entire study population suggests that the distribution of tumor samples within the subgroups was representative of all patients entered into the trial. In support of the NCIC findings, the role of an increase in the numbers of copies of the *EGFR* gene as a potential marker of sensitivity to gefitinib in non–small-cell lung cancer has recently been demonstrated by others.[10]

These results, however, raise critical new questions with regard to the mechanism of action of erlotinib in non–small-cell lung cancer and the methodologic requirements for molecular pharmacodynamic studies in the future. Although increases in the number of gene copies, protein expression, and *EGFR* mutations were associated with a response, objective responses to erlotinib were also found in the absence of these features. This finding suggests that erlotinib may affect, or be dependent for its activity on, additional signaling pathways that were not assessed in the NCIC study (such as Akt phosphorylation or erbB-3 status) and that are essential for tumor-cell proliferation.[10,11] Thus, further investigation to determine the mechanisms by which erlotinib alters the full range of EGFR-related signaling pathways is essential and is likely to lead to the discovery of a panel of molecular markers that will eventually be used to predict the therapeutic activity of this class of drugs in non–small-cell lung cancer.

Perhaps of greatest importance is the fact that samples of tumor tissues from fewer than half the patients who participated in the NCIC trial were usable for EGFR analysis. In particular, the small number of samples that could be examined for *EGFR* mutations or number of gene copies clearly reduced the strength of the conclusions it was possible to draw from this study. Furthermore, the tissues that were available were taken from either initial surgical specimens or initial or subsequent biopsies performed before the initiation of therapy. In the absence of a standardized approach to ensuring tumor acquisition at entry into the study, the effect of tumor size and time from diagnosis to first treatment on measures of EGFR could not be controlled for in the trial.

With the development of trastuzumab therapy for breast cancer as a model,[12] it is now clear that predictive molecular assays must be devised before the initiation of clinical trials for new targeted anticancer agents if the specificity and usefulness of these drugs are to be meaningfully evaluated in the population of patients most likely to benefit from the treatment. Furthermore, the designs of modern phase 2 and phase 3 clinical trials must incorporate specific molecular assays to maximize the likelihood of definitive clinical results. This work will require substantially more effort to develop standardized assay procedures for assessing and predicting the effects of whole classes of molecularly targeted agents and highly standardized methods for the prospective collection of human tumor tissue.

The results of the NCIC study of erlotinib for non–small-cell lung cancer support the promise that individualized oncologic therapies can be developed and applied on the basis of the specific molecular characteristics of a patient's malignant disease. Only the most assiduous attention to the development of drug-specific molecular pharmacodynamic tools, and the implementation of meticulous tumor-acquisition techniques, will allow that promise to be realized.

From the Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, Md.

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Pacemaker Selection — The Changing Definition of Physiologic Pacing

Kenneth A. Ellenbogen, M.D., and Mark A. Wood, M.D.

Almost 50 years after the first report of the use of cardiac pacing, in the *Journal*,[1] pacing is playing an increasingly important role in the management of cardiac disease. Cardiologists have evaluated single-chamber, dual-chamber, and triple-chamber pacemakers in patients with different types of conduction-system disease and underlying cardiac function. The goal of these increasingly complex pacing systems is to reproduce the normal electrical activation of the heart.

Dual-chamber pacing confers potentially important hemodynamic advantages over ventricular pacing by linking the timing of atrial and ventricular contraction, a phenomenon called atrioventricular synchrony.[2] In short-term and long-term studies, atrioventricular synchrony improves stroke volume, raises systolic blood pressure, reduces right atrial pressure and pulmonary-capillary wedge pressure, and is less likely to elicit cardioinhibitory reflexes than is ventricular pacing. The importance of the atrial contribution to cardiac output has been demonstrated in a variety of patient groups as well as at different heart rates. The restoration of atrioventricular synchrony by pacing was branded early on as "physiologic pacing" because it mimics the normal sequence of atrioventricular activation. The expectation was that the hemodynamic benefits of atrioventricular synchrony would translate into reductions in cardiac mortality, a reduced risk of heart failure, and a better quality of life.

These expectations have been examined in two previous large, randomized clinical trials comparing dual-chamber pacing with single-chamber pacing. These trials, predominantly involving patients with the sick sinus syndrome and having a combined enrollment of more than 4500 patients, showed a relative reduction in the risk of atrial fibrillation that ranged from 18 to 23 percent.[3,4] No difference in total mortality, mortality from cardiovascular causes, or stroke was observed.

It was only natural that a similar evaluation would be conducted in patients with high-grade atrioventricular block, who make up a substantial portion of patients now receiving pacemakers. In this issue of the *Journal*, Toff and colleagues report the results of the United Kingdom Pacing and Cardiovascular Events (UKPACE) trial, a comparison of single-chamber pacing with dual-chamber pacing in elderly patients with high-grade atrioventricular block.[5] The patient population studied differs in important ways from that in other clinical trials in that there was a higher mean age (80 years) and higher annual mortality (7.2 to 7.4 percent). Dual-chamber pacing did not reduce the rate of death from all causes or from cardiovascular causes, nor did it decrease the incidence of atrial fibrillation, myocardial infarction, congestive heart failure or stroke, transient ischemic attack, or other thromboembolism. The authors conclude that, in this patient population, there is no additional benefit to dual-chamber pacing as compared with the simpler and easier-to-implant single-chamber ventricular pacemaker.

The UKPACE trial did not examine quality of life, but this is an important issue. The quality of life in

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Pacemaker Selection — The Changing Definition of Physiologic Pacing

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Almost 50 years after the first report of the use of cardiac pacing, in the *Journal*,[1] pacing is playing an increasingly important role in the management of cardiac disease. Cardiologists have evaluated single-chamber, dual-chamber, and triple-chamber pacemakers in patients with different types of conduction-system disease and underlying cardiac function. The goal of these increasingly complex pacing systems is to reproduce the normal electrical activation of the heart.

Dual-chamber pacing confers potentially important hemodynamic advantages over ventricular pacing by linking the timing of atrial and ventricular contraction, a phenomenon called atrioventricular synchrony.[2] In short-term and long-term studies, atrioventricular synchrony improves stroke volume, raises systolic blood pressure, reduces right atrial pressure and pulmonary-capillary wedge pressure, and is less likely to elicit cardioinhibitory reflexes than is ventricular pacing. The importance of the atrial contribution to cardiac output has been demonstrated in a variety of patient groups as well as at different heart rates. The restoration of atrioventricular synchrony by pacing was branded early on as "physiologic pacing" because it mimics the normal sequence of atrioventricular activation. The expectation was that the hemodynamic benefits of atrioventricular synchrony would translate into reductions in cardiac mortality, a reduced risk of heart failure, and a better quality of life.

These expectations have been examined in two previous large, randomized clinical trials comparing dual-chamber pacing with single-chamber pacing. These trials, predominantly involving patients with the sick sinus syndrome and having a combined enrollment of more than 4500 patients, showed a relative reduction in the risk of atrial fibrillation that ranged from 18 to 23 percent.[3,4] No difference in total mortality, mortality from cardiovascular causes, or stroke was observed.

It was only natural that a similar evaluation would be conducted in patients with high-grade atrioventricular block, who make up a substantial portion of patients now receiving pacemakers. In this issue of the *Journal*, Toff and colleagues report the results of the United Kingdom Pacing and Cardiovascular Events (UKPACE) trial, a comparison of single-chamber pacing with dual-chamber pacing in elderly patients with high-grade atrioventricular block.[5] The patient population studied differs in important ways from that in other clinical trials in that there was a higher mean age (80 years) and higher annual mortality (7.2 to 7.4 percent). Dual-chamber pacing did not reduce the rate of death from all causes or from cardiovascular causes, nor did it decrease the incidence of atrial fibrillation, myocardial infarction, congestive heart failure or stroke, transient ischemic attack, or other thromboembolism. The authors conclude that, in this patient population, there is no additional benefit to dual-chamber pacing as compared with the simpler and easier-to-implant single-chamber ventricular pacemaker.

The UKPACE trial did not examine quality of life, but this is an important issue. The quality of life in

patients with pacemakers is often poor and in one trial was found to be similar to that of patients who require long-term hemodialysis.[4] It has been difficult to demonstrate improved quality of life with dual-chamber pacing in the elderly. In the Pacemaker Selection in the Elderly (PASE) trial, in which the primary end point was quality of life, no overall benefit from dual-chamber pacing as compared with single-chamber ventricular pacing was found in an elderly cohort.[6] In a subgroup analysis, patients with sinus-node dysfunction did appear to benefit from dual-chamber pacing, but those with pacemakers implanted because of heart block did not. For the general pacing population, no large trial has yet been reported that used quality of life as the primary end point, although several such trials are in progress.

Dual-chamber pacing does appear to improve the quality of life when comparisons within individual patients, rather than between patients, are made. For example, in a randomized crossover study, Sulke and colleagues compared the effects of different pacing modes on symptoms, functional class, and perceived health status.[7] Single-chamber pacing was the least acceptable pacing mode for 73 percent of patients, and dual-chamber pacing was the preferred mode for 86 percent. Only 9 percent of patients showed no preference for pacing mode, and 5 percent chose single-chamber ventricular pacing. In trials involving the selection of pacing mode in which a dual-chamber pacemaker was implanted, 18 to 26 percent of patients randomly assigned to single-chamber ventricular pacing were unable to tolerate this pacing mode and were switched to a dual-chamber pacing mode. After crossover, patients had significant improvements in quality of life in almost all areas measured.[8] Finally, even patients who have felt generally well for many years with single-chamber pacemakers have noted improvements in their quality of life after being upgraded to dual-chamber pacemakers at the time of a pulse-generator change.[9] Although dual-chamber pacing has not been fully studied, most cardiologists expect that this pacing mode will be shown to improve the quality of life.

The failure to easily demonstrate clinical benefits of dual-chamber pacing has forced a rethinking of what is meant by "physiologic pacing." It is now well accepted that long-term right ventricular pacing causes a deterioration of left ventricular function through complex effects on regional ventricular wall strain and loading conditions. This deterioration is thought to be a result of dyssynchrony between the walls of the left ventricle that is induced by pacing in the right ventricular apex. Sweeney et al. demonstrated, by careful review of data from a large pacing trial, that an increase in the frequency of ventricular pacing in patients with the sick sinus syndrome who had a narrow native QRS complex was associated with an increased incidence of atrial fibrillation and congestive heart failure.[10] These observations were confirmed by Wilkoff et al. in the Dual Chamber and VVI Implantable Defibrillator (DAVID) study, in which backup ventricular pacing and dual-chamber pacing were prospectively compared in patients with dual-chamber defibrillators.[11] The primary end point was a composite of congestive heart failure, hospitalization, and death and was increased by a factor of 1.6 in patients with an increased frequency of ventricular pacing. Thus, right ventricular pacing is a two-edged sword, conferring atrioventricular synchrony but at the same time possibly negating its benefit by inducing ventricular dysfunction.

Cardiac-resynchronization therapy, with pacing of both ventricles as well as of the right atrium (i.e., triple-chamber pacing), has been introduced to compensate for ventricular dyssynchrony. In patients without bradycardia as an indication for pacing but with conduction-system disease and heart failure, cardiac-resynchronization therapy reduces the symptoms of heart failure and improves exercise tolerance, the quality of life, and survival.[12,13] The yet-untested concept is that cardiac-resynchronization therapy, if applied to the general population of people who may benefit from the use of a pacemaker, may overcome the deficiencies of dual-chamber pacing in terms of clinical outcomes.

What can now be said about the selection of patients for dual-chamber, right ventricular pacing? In elderly patients with heart block, dual-chamber pacing will not result in a benefit with respect to hard end points, such as death from cardiovascular disease, heart failure, stroke, or the prevention of atrial fibrillation. Although the expectation of improved survival has been dashed by the results of clinical trials, the question of improvement in the quality of life has not been thoroughly examined. The available evidence suggests that dual-chamber pacing improves the quality of life in at least some patient groups. Furthermore, to the extent that frequent or persistent right ventricular pacing worsens outcomes by inducing left ventricular dysfunction, new pacing algorithms are now available that

lead to a dramatic reduction in the amount of unnecessary ventricular pacing while still preventing ventricular asystole. There are not yet sufficient data to recommend cardiac-resynchronization therapy (i.e., triple-chamber pacing) in patients with bradyarrhythmias who have normal left ventricular function.

It is fair to say that 50 years after the introduction of pacing, this field is undergoing a tremendous revolution. We are learning from both physiology and clinical trials. The best mode of pacing, the best type of pacemaker, and the best position for the pacemaker lead or leads are still not known. What was once heralded as "physiologic" pacing now is regarded in terms of many end points as ineffective at best and deleterious at worst. Much work remains to be done with regard to the development of true physiologic pacing that broadly improves both survival and the quality of life. Cardiologists will have to await the results of ongoing trials involving the quality of life and the further evaluation of triple-chamber pacing systems in a broad range of patients with pacemakers.

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CORRESPONDENCE

Cardiac-Resynchronization Therapy in Heart Failure

TO THE EDITOR: A well-designed study aimed at showing the effect of cardiac resynchronization on mortality among patients with cardiac dyssynchrony, the Cardiac Resynchronization–Heart Failure (CARE-HF) Study (April 14 issue),[1] demonstrated improvements in quality of life and survival well beyond those expected. The question remains as to whether these patients should receive a combination device with both cardioversion and defibrillation capabilities.[2]

Conclusions drawn from a randomized study are very sensitive to the characteristics of the studied population. In CARE-HF, 62 percent of the patients had nonischemic cardiomyopathy. This high percentage raises the question of the generalizability of the results. In a recent meta-analysis of patients with nonischemic cardiomyopathy, Desai et al.[3] concluded that the use of implantable defibrillators reduced mortality. However, their conclusions were based in part on subgroup analyses, which are known to be less reliable than analyses of genuinely randomized study groups. None of the studies that have focused on patients with nonischemic cardiomyopathy have shown a significant reduction in mortality.[3]

Therefore, the impressive effect observed in the CARE-HF Study might not be improved by the addition of a defibrillator in patients with cardiomyopathy. Conversely, the effect of resynchronization on mortality in patients with ischemic cardiomyopathy remains questionable.

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TO THE EDITOR: In the landmark CARE-HF Study, the investigators state that biventricular pacing improves outcomes in patients with cardiac dyssynchrony. This statement is misleading, since only patients with a QRS duration of 120 to 149 msec were required to have echocardiographic evidence of dyssynchrony, and this subgroup made up only 11 percent of the total study population.[1] Thus, the presence of dyssynchrony was not a prerequisite in the majority of patients in this trial, nor was it a prerequisite in any other major study, such as the Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure (COMPANION) trial[2] or the Multicenter InSync Randomized Clinical Evaluation (MIRACLE).[3] In the future, echocardiography may indeed play a role in identifying patients who will not have a response to biventricular pacing, but so far no marker has been universally established as being able to do so. In the meantime, it would be wrong to deny patients who fulfill standard criteria for biventricular pacing a potentially

valuable therapy[4] on the basis of the possibility that they may not have echocardiographic evidence of dyssynchrony.

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THE AUTHORS REPLY: Dr. Burri suggests that current criteria for measuring cardiac dyssynchrony are essentially untested in clinical trials and therefore are inadequate for the selection of patients for cardiac-resynchronization therapy. This interpretation of the existing data is correct but narrow. As with all clinical trials, important questions remain, even when the results are clear.

Atriobiventricular pacing is clinically effective in patients with a high prevalence of dyssynchrony and appears somewhat more effective in those with echocardiographic evidence of interventricular mechanical delay,[1] whereas conventional right ventricular pacing is associated with an adverse outcome in patients with heart failure.[2] These considerations effectively rule out mechanisms other than resynchronization, by which pacing might induce benefit. Many, but not all, patients with a QRS duration of more than 149 msec benefited from cardiac-resynchronization therapy in the CARE-HF Study. Clearly, there is much more to learn about the measurement of cardiac dyssynchrony to ensure optimal use of cardiac-resynchronization therapy, but ultimately it is cardiac dyssynchrony rather than a broad QRS interval that is the treatment target. Dr. Burri's concerns about the adequacy of current measures of dyssynchrony are legitimate. Practical and reliable means of assessing dyssynchrony will be required to identify patients with less severe symptoms who may benefit from this technology.

Dr. Kirkorian suggests that patients with ischemic heart disease may not have benefited from cardiac-resynchronization therapy. In the CARE-HF Study, more than 40 percent of the patients randomly assigned to cardiac-resynchronization therapy had definite coronary disease, and in an additional 25 percent coronary disease was not ruled out.[3] There was no evidence of heterogeneity in outcomes between patients with ischemic heart disease and those without it. It is likely that there is a subgroup of patients with extensive myocardial scarring in whom resynchronization is not satisfactory, but for many patients with ischemic heart disease and dyssynchrony cardiac-resynchronization therapy does appear to be effective. There is no evidence that the relative benefits of implantable defibrillators vary according to the presence or absence of ischemic heart disease.[4,5] Clearly, investigation of the incremental benefit of defibrillators in addition to cardiac-resynchronization therapy in patients with dyssynchrony will be needed to ensure that the safest, most effective, and most cost-effective therapy is being delivered.

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EGFR Mutation Conferring Primary Resistance to Gefitinib in Non–Small-Cell Lung Cancer

TO THE EDITOR: Patients with lung cancer who respond to gefitinib have been reported to have somatic mutations consisting of deletions in exon 19 and the L858R mutation in exon 21 of the epidermal growth factor receptor (*EGFR*) gene.[1] In addition, a second mutation (T790M) in exon 20 is also associated with acquired resistance to gefitinib in initially gefitinib-sensitive patients.[2,3] We describe a patient with gefitinib-resistant lung adenocarcinoma harboring both T790M and L858R at diagnosis.

A 55-year-old woman who had never smoked presented with blurred vision and slurred speech. Magnetic resonance imaging of the brain disclosed a rim-enhanced mass in the left parietal–occipital area. Computed tomography of the chest showed a mass in the right upper lung with enlarged lymph nodes in the lower neck and mediastinum. Percutaneous transthoracic biopsy guided by ultrasonography revealed lung adenocarcinoma. Gefitinib was started at a dose of 250 mg per day. The patient also underwent whole-brain radiotherapy and stereotactic radiosurgery for control of the brain tumor. One month later, the size of the lung tumor was unchanged, but at nine weeks, chest radiography revealed progression of disease. Gefitinib was stopped, and treatment was changed to chemotherapy with gemcitabine and cisplatin.

Screening for mutations of the kinase domain (exons 18 through 21) of *EGFR* by direct sequencing of DNA isolated from a lung-tumor–biopsy specimen and blood lymphocytes identified a T-to-G mutation at nucleotide 2573 of exon 21, resulting in L858R (Fig. 1A). A C-to-T mutation was identified at nucleotide 2369 of exon 20, resulting in T790M (Fig. 1B). The mutations were detected in both sense and antisense sequences of two independent polymerase chain reactions and were confirmed by subcloning.

Lung cancers harboring the *EGFR* L858R mutation have been reported to be responsive to gefitinib.[1] The studies of cells expressing L858R revealed increased gefitinib sensitivity in vitro.[4] The

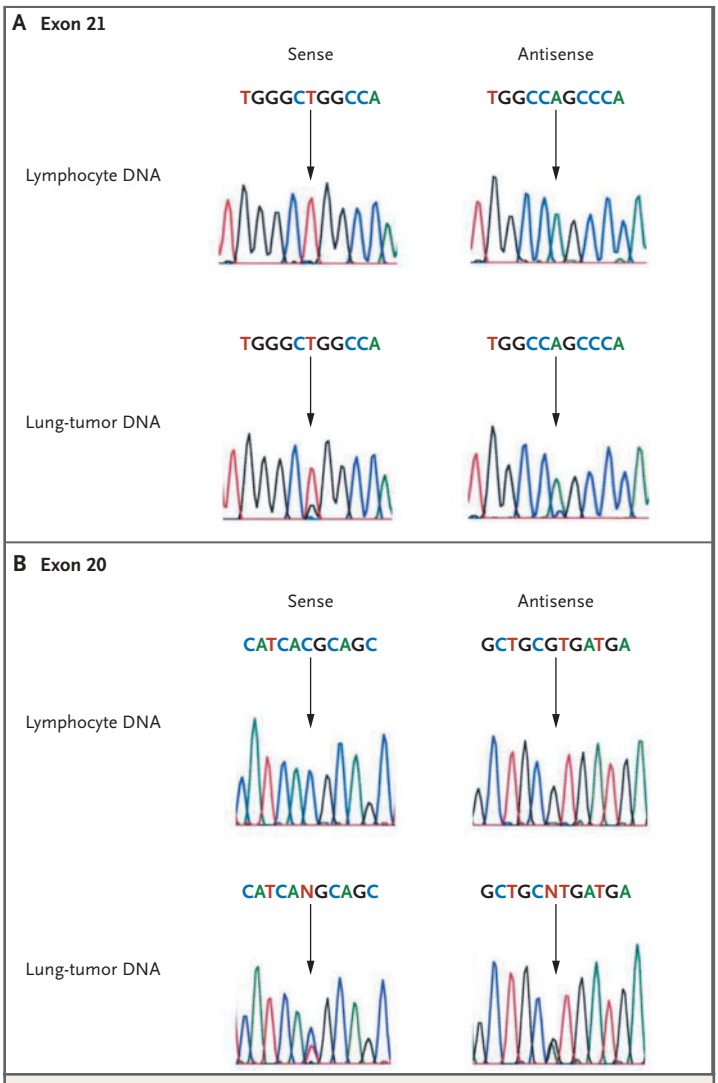

Figure 1. Sequencing Chromatograms with *EGFR* Mutations in the Tumor-Biopsy Specimen at Diagnosis of Lung Adenocarcinoma.

In Panel A, sequencing of exon 21 shows a T-to-G (antisense, A-to-C) base-pair change (arrows), resulting in a substitution of arginine for leucine at position 858 (L858R) in the tumor, as compared with corresponding lymphocytes. In Panel B, sequencing of exon 20 demonstrates a C-to-T (antisense, G-to-A) base-pair change (arrows), which leads to the substitution of methionine for threonine at position 790 (T790M). The ratio of mutant to wild-type peaks is approximately 1:1; therefore, the nucleotide sequence is read as N.

T790M mutation was shown to confer resistance to gefitinib after it was introduced into the sequence of the wild-type *EGFR* and L858R mutant *EGFR* in vitro.[2,3] The T790M mutation results in steric hindrance of binding of gefitinib to the ATP-kinase–binding pocket. The T790M mutation may cause acquired resistance to gefitinib.[2,3] Our patient had concomitant T790M and L858R *EGFR* mutations in the original lung-biopsy specimen and showed primary resistance to gefitinib — a finding implying that a mutation in the T790M kinase domain can occur during the natural evolution of lung cancer. T790M mutant gefitinib-resistant clones may preexist at levels below the threshold of detection[5] in some patients with lung cancer at presentation and then may expand selectively under gefitinib treatment, leading to the failure of gefitinib therapy.

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Responsiveness to Cetuximab without Mutations in *EGFR*

TO THE EDITOR: A large amount of information suggests that mutations in the kinase domain of epidermal growth factor receptor (EGFR) are critical for the efficacy of EGFR kinase inhibitors.[1-3] However, the effect of *EGFR* mutations on the response to cetuximab has not been directly investigated. Barber et al.[4] reported the absence of *EGFR* mutations in colorectal cancers and speculated that *EGFR* mutations were not required for the response to cetuximab, since it was an efficacious agent against this type of tumor.[5] We sequenced the kinase domain of *EGFR* (exons 18, 19, and 21) in tumor samples from 38 patients participating in a cetuximab-monotherapy study for recurrent non–small-cell lung cancer and tumor samples from 39 patients participating in a cetuximab-monotherapy study for refractory colorectal cancer. Mutations previously detected in non–small-cell lung cancer[1-3] were identified in 3 of the 38 patients with non–small-cell lung cancer. Of 13 patients with non–small-cell lung cancer whose disease was stable, 2 carried a del746-750, and of 21 patients with progressive disease, 1 had an L861Q mutation. No mutations were identified in other patients with non–small-cell lung cancer who had a partial response (one patient) or for whom response data were unavailable (three patients). No mutations were detected in the samples from the 39 patients with colorectal cancer, including those from 20 patients who had a partial response and 1 who had a complete response.

From these results, it appears that the presence of an *EGFR* mutation is not a major determining factor for a positive response to cetuximab. Absence of an *EGFR* mutation in the samples of colorectal cancer, including those from patients who had a response to cetuximab, supports the speculation by Barber et al.[4] that *EGFR* mutations are not required for the efficacy of cetuximab in colorectal cancer. (Some of the samples were chosen for sequence analysis on the basis of the clinical response, and thus the numbers we mention in this letter do not reflect the response rates in the trials.) In addition, we sequenced 160 biopsy samples of previously untreated colorectal cancer (provided by Dr. Sina Dorudi, Royal London Hospital, London) from patients outside the cetuximab trial and could not identify any mutation in exons 18, 19, and 21. This further confirms the general absence of *EGFR* mutations in colorectal cancer. Our results suggest

that in contrast to the correlation of mutations with responses to the *EGFR* kinase inhibitors gefitinib and erlotinib, *EGFR* mutations are not critical for the response of a tumor to cetuximab.

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Lack of Mutations in *EGFR* in Gastroenteropancreatic Neuroendocrine Tumors

TO THE EDITOR: Lynch et al.[1] and Paez et al.[2] correlated the presence of somatic mutations in the tyrosine kinase domain of the gene that encodes the epidermal growth factor receptor (*EGFR*) with responsiveness to gefitinib in non–small-cell lung carcinoma. Neuroendocrine differentiation is present in a substantial number of gastroenteropancreatic neuroendocrine tumors,[3] and EGFR expression occurs in such tumors.[4]

We sought EGFR-activating mutations in a series of rare gastroenteropancreatic neuroendocrine cancers. DNA was isolated from flash-frozen specimens of carcinoid and pancreatic endocrine tumors; *EGFR* exons 18, 19, and 21 were amplified by the polymerase chain reaction with primers described by Lynch et al.[1]; and amplicons were sequenced and assessed for predictive *EGFR* mutations (single-nucleotide substitutions in exons 18 and 21 and in-frame deletions in exon 19). Research authorization was obtained from all the patients, and the study was approved by the institutional review board.

No mutations in the *EGFR* kinase domain that were predictive of a response to gefitinib were detected in DNA from 62 human carcinoid tumors from a variety of sites, including both primary lesions (22 from the lung, 28 from the ileum, and 1 from the colon) and metastatic lesions (30 from the liver and 1 from the ovary). Most carcinoid tumors in this study were indolent; 20 matched sets, which included an ileal primary lesion and a hepatic metastatic lesion from the same patient, were examined.

As a verification of our detection procedure, DNA controls encoding *EGFR* mutations L858R within exon 21, delE746-A750 within exon 19, and delL747-P753insS within exon 19 (gifts from Drs. Daphne Bell and Daniel Haber, Massachusetts General Hospital, Charlestown, Mass.) were correctly identified. We noted in one ileal carcinoid the reported germ-line synonymous coding-region single-nucleotide polymorphism C/T in *EGFR* exon 21 (dbSNP:2229066; nucleotide position 2694 in GenBank accession number X00588); it was also detected in normal surrounding tissue. Similarly, no mutations in *EGFR* exons 18, 19, and 21 were found in DNA from 18 primary pancreatic endocrine carcinomas. In contrast to our results, Lynch et al.[1] found no mutations in *EGFR* exons 19 and 21 in 40 primary pancreatic tumors (we do not know whether this group included rare pancreatic endocrine tumors) and one bronchial-carcinoid tumor-cell line.

Our results indicate that somatic activating mutations of the *EGFR* kinase domain that are predictive of responsiveness to gefitinib are uncommon in gastroenteropancreatic neuroendocrine cancers, pancreatic endocrine carcinomas, and carcinoid tumors (primary as well as metastatic lesions).

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BOOK REVIEWS

ATHEROTHROMBOSIS AND CORONARY ARTERY DISEASE

Second edition. Edited by Valentin Fuster, Eric J. Topol, and Elizabeth G. Nabel. 1636 pp., illustrated. Philadelphia, Lippincott Williams & Wilkins, 2005. $149. ISBN 0-7817-3583-1.

Most of us walk around oblivious to the time bombs that line our arteries. Indeed, atherosclerosis is, for the most part, a rather benign condition that affects, to some degree, virtually all the adult population both in developed countries and in many developing countries. Many adults will die from other causes before atherosclerosis causes overt problems. If the plaque is big enough and sitting in a coronary artery, it may impede blood flow and cause symptoms of angina. But if it is small, the plaque will usually be asymptomatic until the moment it supports thrombosis. The central role of thrombosis in the evolution of atherosclerotic plaque and the denouement in an acute cardiovascular event are well reflected in the title of this impressive book, and the interplay between plaque and thrombus is one of its central themes.

Medical interest in atherosclerosis stretches back at least four centuries, and the book starts with a fascinating history of the disease that should be of interest to many. The mention of Heberden's classic description of angina pectoris reminds us that angina was a common condition in 18th-century London, and W. Bruce Fye's exhortation to reflect on the question, "Where does the review of the literature end and history begin?" — which Fye poses in the first chapter — provides the perfect start to a book that is intended to present the latest views in an area of fast-moving research. The following chapters cover the science of the disease and clinical practice, and the reader can find illustrations ranging from meta-analyses and Kaplan–Meier survival curves to schematic representations of a model of ATP-binding cassette transporters.

Indeed, one of the greatest strengths of this book is the breadth of what is covered, and by selecting some of the leaders in the field as authors, the editors have provided the necessary authority to the writing, even if styles change from section to section. Breadth is important in an era in which doctoral students frequently find themselves working on highly focused projects dissecting a small piece of the puzzle. Even within a small area, subspecialization is becoming increasingly common. I shudder to recall the graduate student who, when asked in an examination how a blood vessel in a particular experiment was contracted before vasorelaxant agents were available, responded, "But my thesis is about dilatation, not contraction." Reestablishing an integrated approach to understanding atherosclerosis and coronary artery disease is clearly important, and this book should be available in any laboratory working in the field. The chapter on mouse models of atherosclerosis will be of value to many.

There is also a lot here for clinicians: clear and helpful explanations of pathophysiology, a dip into more controversial areas such as the effects of viruses on atherothrombosis, and informative sections on current therapeutic approaches. Flipping back and forth between the section on therapeutics and the historical perspective should help to keep the reader from becoming too convinced that we know exactly how to manage our patients' conditions, even in 2005.

So, are there any problems? Of course, there are some inconsistencies and some omissions: Why, for example, is the important side effect of nocturia caused by calcium-channel blockers never mentioned? These minor flaws are not enough to detract from the quality and importance of the book, but I do have a gripe about the quality of the figures. We increasingly depend on visual images in order to learn, and yet the book is packed with rather dull black-and-white images. Some of those in chapter 19 even look as though they were poorly scanned or downloaded. The wonderful color plates in the middle of the book show what can be used, and it would have been nice to have figures of this quality throughout the book. The most important question, however, is whether I would buy this book. The answer is yes.

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HIGH-RISK ATHEROSCLEROTIC PLAQUES: MECHANISMS, IMAGING, MODELS, AND THERAPY

Edited by Levon Michael Khachigian. 208 pp., illustrated. Boca Raton, Fla., CRC Press, 2005. $99.95. ISBN 0-8493-3028-9.

THE FACT THAT CARDIOVASCULAR DISEASE, for the most part due to atherosclerosis, is the principal killer in Western societies is well known. However, how and why atherosclerotic plaques that develop over many decades rupture in an often unheralded manner — triggering the clinical presentation of an acute coronary syndrome — is still puzzling. *High-Risk Atherosclerotic Plaques* addresses this problem in a wide-ranging manner with chapters that follow a rational sequence, unfolding the processes involved in plaque rupture, reviewing the efforts to develop animal models of the phenomenon, explaining the different methods devised to identify and study vulnerable plaques, and summarizing the current and future strategies for treatment. This book is best suited for readers familiar with basic concepts about the development and clinical presentation of atherosclerosis who seek a detailed review of the mechanisms leading to the presentation of acute coronary syndromes.

The chapters on pathophysiology review important concepts, some of which are essential for the understanding of acute coronary syndromes. For example, there is appropriate emphasis on the concept that "vulnerable plaque" is a prospective term, used to define a local process that occurs as a consequence of many pathophysiological phenomena throughout the coronary tree. The term "vulnerable patient" may be more accurate to describe the systemic nature of the condition and to generate potential therapeutic methods that could modify the disease process. In general, the description in the book of the pathogenetic mechanisms of plaque rupture is thorough and clear. In some instances, by contrast, the techniques developed to study vulnerable plaques — including intravascular ultrasonography, optical coherence tomography, magnetic resonance imaging, and nuclear imaging — are laid out in unnecessary detail, particularly in the context of a book that focuses on applications, rather than details, of techniques. Nevertheless, these chapters provide the reader with tools to understand the advantages and limitations of these techniques, especially in relation to their investigational use for the identification of changes induced by treatment. Less clear is the pragmatic role these techniques may have in clinical practice, since prospective identification of vulnerable plaques would require screening vast numbers of people, each one serially, for long periods of time. The last chapter, on therapeutic strategies, is comprehensive and up to date and is perhaps one of the pearls of the book.

There are a few signs of poor editing. Similar concepts related to pathophysiology repeated in different chapters become redundant, and two tables containing the same information are included. A chapter on systemic markers of inflammation (e.g., C-reactive protein) would have been desirable, particularly since inflammation is an important component of the pathophysiology and the vulnerable plaque is part of a systemic condition.

The quest to understand and halt the processes that convert a previously quiescent atherosclerotic plaque into one responsible for untoward clinical outcomes is one of the most exciting and potentially rewarding pursuits in cardiovascular medicine. Overall, this book represents a valuable tool for both the novice and the scholar who are interested in this topic and provides an excellent source of reference material.

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HEART FAILURE: A COMPREHENSIVE GUIDE TO DIAGNOSIS AND TREATMENT

(Fundamental and Clinical Cardiology. Volume 50.) Edited by G. William Dec, Thomas DiSalvo, Roger J. Hajjar, and Marc J. Semigran. 582 pp., illustrated. New York, Marcel Dekker, 2005. $179.95. ISBN 0-8247-5827-7.

SIR THOMAS LEWIS OPINED IN 1933 THAT "the very essence of cardiovascular practice is the early detection of heart failure." Heart failure at that time was a topic of substantial interest to physicians. In the past two decades that interest has been revived, and no longer is cardiology overlooked in favor of surgery, catheter intervention, and the early management of acute coronary syndromes. This change has come about because of the realization that heart failure is a common cardiac prob-

lem that is becoming more common, is detectable in the community, is preventable, is treatable, and consumes a considerable proportion of health care resources.

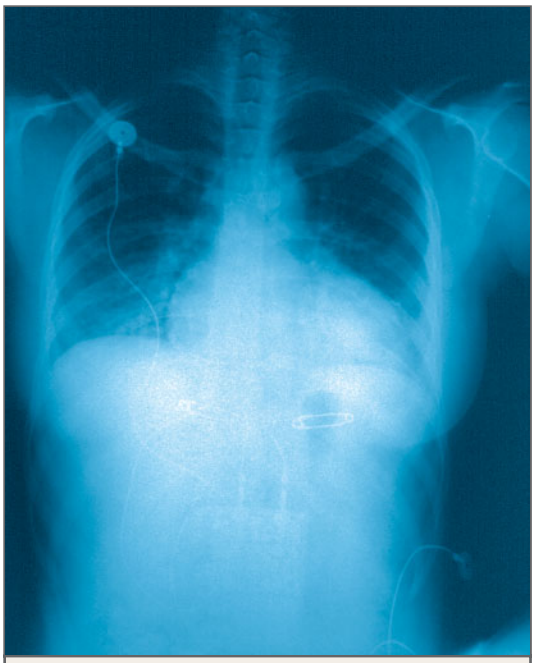
Heart failure is an illness of major public importance, and new treatments, such as the use of inhibitors of the renin–angiotensin and sympathetic systems, have been shown to be efficacious in large, high-quality trials. Indeed, in few branches of medicine has it been shown so clearly how new drugs affect key outcomes — namely, death and the quality of life. The current emphasis is on methods to encourage the implementation of this new medical knowledge. At the same time, many ideas are emerging concerning the prevention of the progression of heart failure or the reversal of the fundamental cause of heart failure — the loss of functioning myocytes. In the immediate future, advances in the fields of engineering and electronics are likely to contribute considerably to cardiac-resynchronization treatment, implantable cardioverter–defibrillators, and left ventricular assist devices. In the longer term, the cure for heart failure must be control of the growth, death, regeneration, and perhaps even cell division of the cardiac myocytes.

This book is a contribution to the ever-growing number of works being published on the topic of heart failure. It is written by a distinguished set of authors, has 25 chapters, is eminently readable, and is of a weight that makes it easy to take off the shelf. It is well referenced, perhaps overly so, and is up to date. There are outstanding chapters on beta-adrenergic signaling, excitation–contraction coupling, heart failure in special populations, angiotensin-receptor blockers, and new treatments such as mechanical support, immunotherapy, gene therapy, and cell treatment. There is useful information on genetic diversity, and it is perhaps a pity that there is no specific chapter on that topic. The review of heart failure in women, the elderly, and ethnic groups is excellent and identifies deficiencies in current knowledge.

The worst feature of the book by far is the quality of the figures, some of which are simply awful. Omitted is a focus on the delivery of health care in the community, the use of health care specialists and nurses for the delivery of that care, and the following of guidelines by members of the community. The problem is that most clinical trials have reported on the use of treatments in men in their 60s, even though the average age of persons with heart failure in Western societies is 75 years and the elderly population is nearly 50 percent female.

The authors adhere rather strictly to the principles of evidenced-based medicine, but not always. For example, there is little evidence that beta-blockers are of benefit in patients who have structural heart disease without symptoms, although it is a reasonable supposition. A long discussion about the use of digoxin is rather unbalanced; the author has a committed belief in the merits of the drug. Perhaps the general point is that when guidelines committees try to assess the outcomes of large trials and the effect on health systems, they should not only identify positive trials but also emphasize neutral or even negative trials.

The recent guidelines of the American Heart Association and the American College of Cardiology have introduced a new classification of heart failure that is based on four stages. Stage A includes patients at high risk with no structural disorders, stage B patients with structural disease and no symptoms, stage C patients with structural disease and symptoms, and stage D patients with severe heart failure



Radiograph Showing Congestive Heart Failure in a 28-Year-Old Woman.

Courtesy of the Centers for Disease Control and Prevention/Dr. Thomas Hooten.

or end-stage disease. This system contrasts with the widely used classification from the New York Heart Association, in which grades 1 through 4 are based on the severity of symptoms. The change is a consequence of a better understanding of the biology of heart failure, of the importance of inhibiting the progression of heart failure, and in particular, of the importance of preserving cardiac myocytes and preventing progressive enlargement of the ventricle (i.e., remodeling). This book is a most useful summary of the current state of knowledge in the field of heart failure; many readers will gain from dipping into it.

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NOTICES

Notices submitted for publication should contain a mailing address and telephone number of a contact person or department. We regret that we are unable to publish all notices received. Notices also appear on the Journal*'s Web site (www.nejm.org/meetings). The listings can be viewed in their entirety or searched by location, month, or key word.*

WOMEN WITH DISABILITIES SYMPOSIUM

The symposium, entitled "A Practical Approach to Providing Quality Care to Women with Visual, Hearing, and Mobility Impairments," will be held in Boston on Sept. 17. It is presented by Harvard Medical School's Center of Excellence in Women's Health and co-sponsored by the Center for Women with Disabilities at Beth Israel Deaconess Medical Center.

Contact Lindsay Giorgi, Harvard Medical School's Center of Excellence in Women's Health, 1620 Tremont St., Boston, MA 02120; or call (617) 732-5502; or e-mail lgiorgi@partners.org.

CANCER MEDICINE AND HEMATOLOGY

The course will be offered in Boston, Sept. 18–23. It is jointly presented by the Dana–Farber Cancer Institute, Brigham and Women's Hospital, and Massachusetts General Hospital.

Contact Harvard Medical School, Department of CME, P.O. Box 825, Boston, MA 02117-0825; or call (617) 384-8600; or fax (617) 384-8686; or e-mail hms-cme@hms.harvard.edu; or see http://www.cme.hms.harvard.edu.

CLEVELAND CLINIC FOUNDATION

The "41st Annual Gastroenterology Update" will be offered in Cleveland, Oct. 6 and 7. It is jointly sponsored by the Cleveland Clinic Department of Gastroenterology and Hepatology and the American College of Gastroenterology.

Contact The Cleveland Clinic Center for Continuing Education, 9500 Euclid Ave., Cleveland, OH 44195; or call (800) 762-8173 (national) or (216) 444-5696 (Ohio); or fax (216) 445-9406; or see http://www.clevelandclinicmeded.com/registration.htm.

16TH INTERNATIONAL SYMPOSIUM ON THE AUTONOMIC NERVOUS SYSTEM

The symposium will be held in Los Cabos, Mexico, Oct. 6–9.

Contact Anita Zeller, American Autonomic Society, 18915 Inca Ave., Lakeville, MN 55044; or call (952) 469-5837; or fax (952) 469-8424; or see http://www.americanautonomicsociety.org; or e-mail zeller.anita@mayo.edu.

16TH CONGRESS OF THE EUROPEAN SOCIETY OF AMBULATORY PEDIATRICS

The congress will be held in Barcelona, Oct. 7 and 8.

Contact Muriel Idelsohn, Congress Department, Grupo Pacifico, Maria Cubi, 4 Pral., 08006 Barcelona, Spain; or call (34) 932 388 777; or e-mail midelsohn@pacifico-meetings.com; or fax (34) 932 387 488; or see http://www.pacifico-meetings.com.

11TH WORLD CONGRESS ON THE MENOPAUSE

The congress will be held in Buenos Aires, Oct. 18–22.

Contact Congress Secretariat, Ana Juan Congresos, Malasia 884, C1426BNB Buenos Aires, Argentina; or call (54) 11 4381 1777; or fax (54) 11 4382 6703; or e-mail menopause@anajuan.com; or see http://www.anajuan.com/menopause.

EUROPEAN SOCIETY OF PAEDIATRIC AND NEONATAL INTENSIVE CARE

The "16th ESPNIC Medical and Nursing Annual Congress," entitled "Paediatric and Neonatal Care: Changing the Paradigm," will be held in Antwerp, Belgium, Sept. 15–17.

Contact Congress Secretariat, Kenes International, 17 rue du Cendrier, P.O. Box 1726, CH-1211 Geneva 1, Switzerland; or call (41) 22 908 0488; or fax (41) 22 732 2850; or e-mail espnic@kenes.com; or see http://kenes.com/espnic.

TRANSPLANT IMMUNOSUPPRESSION 2005: IMPROVING RECIPIENT OUTCOME

The congress will be held in Minneapolis, Sept. 28–Oct. 1.

Contact Office of CME, University of Minnesota, 190 McNamara Alumni Center, 200 Oak St. SE, Minneapolis, MN 55455; or call (800) 776-8636 (national) or (612) 626-7600 (Minnesota); or fax (612) 626-7766; or see http://www.cme.umn.edu.

BOSTON UNIVERSITY SCHOOL OF MEDICINE

The following courses will be offered: "Cardiology for the Consulting Cardiologist: Balancing Contemporary Medical Therapy with Emerging Interventions" (Boston, Oct. 20 and 21) and "Pediatric Infectious Diseases in the Headlines" (Cambridge, Mass., Oct. 28 and 29).

Contact Department of CME, Boston University School of Medicine, 715 Albany St., A305, Boston, MA 02118-2526; or call (617) 638-4605; or fax (617) 638-4905; or e-mail cme@bu.edu; or see http://www.bu.edu/cme.

15TH ANNUAL IRWIN M. ARIAS, M.D., SYMPOSIUM

The symposium, entitled "Bridging Basic Science and Liver Disease," will be held in Boston on Sept. 22.

Contact American Liver Foundation New England Chapter, 88 Winchester St., Newton, MA 02461; or call (617) 527-5600; or fax (617) 527-5636; or see http://www.liverfoundation-ne.org.

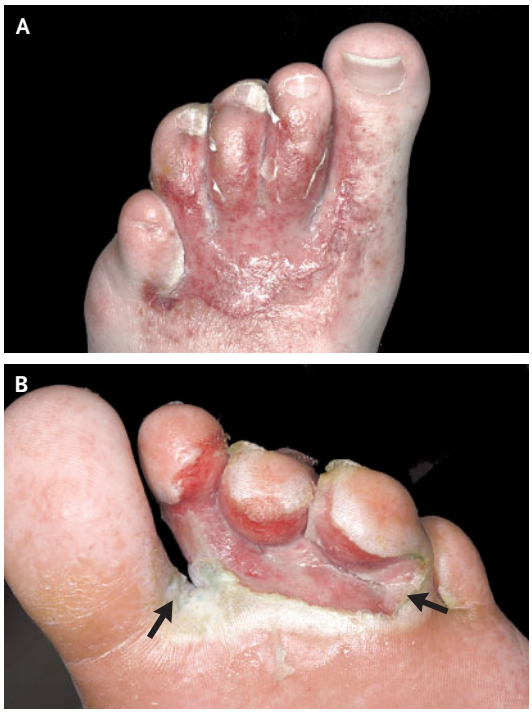
ROOSEVELT ISLAND HISTORICAL SOCIETY

The Roosevelt Island Historical Society is seeking physicians who worked on the New York City island as students, interns, and residents to learn about their experiences. The island was known as Welfare Island until 1973.

Contact Judith Berdy, Roosevelt Island Historical Society, 575 Main St., Roosevelt Island, NY 10044; or call (212) 688-4836; or e-mail rooseveltislandhistory@usa.com.

IMAGES IN CLINICAL MEDICINE

Pseudomonas Cellulitis



Thomas P. Habif, M.D.

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A healthy 42-year-old man presented with a broad eroded, ulcerated lesion on the left foot, which had developed over the previous month. His new job required that he wear boots, which caused his feet to perspire. Toe-web scale accumulated and became moist. Erythema and vesicles appeared in the webs and then extended onto the dorsum. Itching became intense. The patient applied a moderately strong topical steroid, which provided temporary relief, but then the eruption became painful and much more extensive.

Examination showed an ulcerated area on the dorsal (Panel A) and plantar surfaces. The skin around the erosions was stained green (Panel B, arrows). A culture was positive for *Pseudomonas aeruginosa*. The patient was treated with acetic acid (diluted vinegar) in wet compresses, ciclopirox in a topical suspension, and levofloxacin, administered orally, for 10 days. All areas had healed four weeks later, at which time the patient was able to return to work. The importance of keeping his feet dry when wearing boots was reinforced.