Gene Signals Relapse of Breast, Ovarian Cancers

Two years of controversy over an early-warning sign of cancer relapse appear to be coming to an end

TWO YEARS AGO DENNIS SLAMON of the UCLA School of Medicine and his colleagues thought that they had found a valuable new tool for predicting the prospects of breast cancer patients. Trouble was, not everyone could confirm the Slamon group's results.

The simmering controversy now appears to be coming to an end. On page 707 of this issue of *Science*, Slamon and his colleagues report the results of an expanded study of breast cancer patients that confirms their original conclusion. Other groups are also weighing in with new confirmatory data.

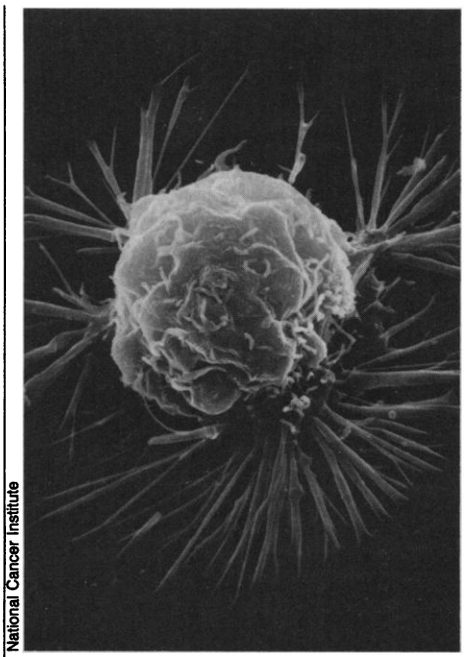
Not only that, but in the ensuing 2 years, the Slamon group has extended its findings to ovarian cancer, thereby providing a possible biological link between two cancers that together account for one-third of all new malignancies in women and one-quarter of all the cancer deaths.

At the very least, the research may lead to improved treatment of breast and ovarian cancers by helping physicians identify those patients who need aggressive therapy to prevent relapses after their original tumors are removed surgically. But it may also make it possible to design novel chemotherapeutic drugs for preventing relapses in the patients.

What Slamon and his colleagues originally observed in 1987, and have now confirmed, is a genetic signal in tumors from breast cancer patients who have poor prognoses. That signal comes in the form of an oncogene, variously known as *neu*, *HER-2*, or *erbB-2*, that appears in multiple copies in tumors from women who are more likely to suffer a relapse and die early.

Although some groups confirmed this correlation between *neu* gene amplification and a poor prognosis, others did not. The discrepant results may have been attributable, Slamon says, to the fact that the various groups used different methods of assessing the gene amplification. Some looked for extra copies of the gene DNA; others for increased expression in the form of higher than normal amounts of the corresponding messenger RNA or the *neu* protein itself. The results given by the different techniques may not have been strictly comparable.

Another problem is the variable composition of breast tumor tissue. Cancer cells may make up only 50% of the total, and the presence of DNA and other normal cell constituents may mask abnormalities in the cancer cells.



National Cancer Institute

A human breast cancer cell. *If the* neu *gene is amplified, it may grow faster.*

Also important was the relatively small size of the early studies on *neu* amplification as a prognostic indicator. There was a general consensus that none included sufficient patients to give a definitive answer about what the extra *neu* gene copies might mean for a breast cancer patient. "To make a long story short," Slamon says, "we had to do a study with a larger number of patients. We found that what we originally observed is true."

The current study included 526 patients—more than five times the number in the original trial—whose long-term outcomes were known. In addition, wherever possible, Slamon and his colleagues assessed all aspects of *neu* gene amplification and expression.

As before, the researchers found the *neu* gene to be amplified in tumors from about 25% of the women. The presence of extra copies of the gene was always accompanied by signs of increased expression of the gene in the tumors.

The amplification correlated with a bad prognosis for patients whose cancers had already spread to the underarm lymph nodes, but not for patients whose lymph nodes were still free of cancer cells. Failure to find a correlation for these node-negative patients may have been due, Slamon says, to the small number of relapses occurring in this group, which constituted only about a third of the total patients in the study.

Other recent studies have found that the correlation holds true for node-negative, as well as node-positive, patients, however. These include one reported in the 15 April issue of *Cancer Research* by Christopher Wright, Adrian Harris, and their colleagues at the Imperial Cancer Research Fund's Clinical Oncology Unit in Oxford, England, and also another study, which is headed by breast cancer specialist Marc Lippman of Georgetown University School of Medicine in Washington, D.C. "The data that I have seen all suggest that this [gene amplification] is a major prognostic indicator," Lippman says.

Malcolm Patterson of the Cross Cancer Institute in Edmonton, Alberta, who collaborates with Slamon, has looked only at tumors from node-negative women and also finds that the correlation holds. "What is striking," Patterson says, "is that patients in whose tumor specimens the [*neu* gene] DNA was amplified more than six times all relapsed in less than 36 months."

Having good prognostic indicators for node-negative women is especially important. In the past, these patients have not usually been given chemotherapy because they have much lower risk of having a recurrence of their cancer than patients with lymph node metastases. Nevertheless, a significant minority—about 30%—of the node-negative group relapse within 5 years. Clinical trials indicating that chemotherapeutic drugs can reduce this relapse rate led officials of the National Cancer Institute to recommend last year that all node-negative women receive the drugs.

Chemotherapeutic drugs can have serious side effects, however, and not everyone agrees that their benefits justify the risks of giving them to all node-negative women. But if the women who are likely to have recurrences of their cancers could be identified reliably, the others could be spared the rigors of additional therapy.

According to Slamon, there are currently no good prognostic indicators for ovarian cancer. Most women with this malignancy already have metastases by the time their disease is diagnosed. The discovery that *neu*

gene amplification predicts a bad prognosis for these patients may help to guide their treatment. "The ovarian results are even more interesting," Lippman says. "I believe that if they are true, it will be extremely useful."

Ovarian and breast cancer have a number of common features. The female hormone estrogen influences the growth of both. Moreover, women who have one cancer are at increased risk of developing the other. The Slamon group's results now suggest that the same cellular derangements may contribute to the development of the two cancers.

Whether the *neu* gene amplification plays a causative role in the two human cancers remains to be established, but work with animals and cultured cells suggests that it may. For example, Philip Leder, William Muller, and their colleagues at Harvard Medical School and the Howard Hughes Medical Institute have introduced the active *neu* oncogene into mice. Expression of the transferred gene in the mouse mammary tissue is sufficient by itself to produce malignant mammary tumors in the animals.

Leder initiated these experiments as part of his continuing investigations of oncogene action and before the link between *neu* gene amplification and breast cancer prognosis was made. "But," he notes, "that correlation when it came made *neu* an extremely interesting oncogene in breast cancer.

Other investigators, including the NCI's Stuart Aaronson and Axel Ullrich of Genentech, Inc., in South San Francisco, have shown that overproduction of the protein encoded by the normal *neu* gene can give cultured cells malignant properties. As mentioned, the human breast cancers cells in which the gene is amplified make higher than normal amounts of the *neu* protein. So do the ovarian cancer cells.

Overproduction of the *neu* protein might cause tumor cells to behave aggressively by causing them to grow faster than they otherwise would. The *neu* protein has all the earmarks of a growth factor receptor. It is structurally similar to the product of another oncogene, called *erbB-1*, which encodes the receptor for epidermal growth factor. No one has yet identified the growth factor that activates *neu* protein, however.

Amplification of the *neu* gene is not the only genetic change that has been implicated in the etiology of breast cancer and may have prognostic value. "We're not saying that this is the only important gene in breast cancer," Slamon remarks. Nevertheless, *neu* gene amplification is turning out after all to be a reliable guide to the prognosis of breast cancer patients, and perhaps of ovarian cancer patients as well. ■ JEAN L. MARX

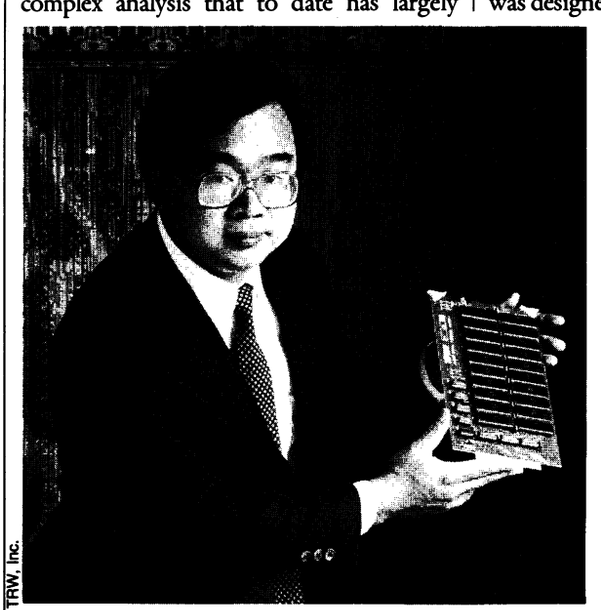
New Chip May Speed Genome Analysis

An unlikely marriage between a defense contractor and Leroy Hood's DNA lab at Caltech is providing a powerful new tool for analyzing complex biological patterns

JUST AS MOLECULAR BIOLOGISTS are becoming buried in data, computer scientists are offering a shovel. DNA sequence data are pouring in as labs around the world gear up to tackle the human genome and other genomes. So far, some 30 million nucleotide bases have been sequenced, and that number is growing by about 10 million bases a year. But getting the complete DNA sequence—the ultimate goal of the human gemome project—is the easy part; deciphering it is a far trickier task. Now help may be in sight from a new computer chip, originally designed for the Defense Department.

Last week Applied Biosystems, Inc., of Foster City, California, announced that it had obtained an exclusive license to this chip, heralded as the "world's fastest text scanning technology," from TRW, Inc., a collaboration that stemmed from work in Leroy Hood's Caltech laboratory, one of 11 National Science Foundation Science and Technology Centers.

It holds out the tantalizing prospect that molecular biologists will soon be able to do at their workstation computers the type of complex analysis that to date has largely been limited to supercomputers—and to do so hundreds of times faster and at a fraction of the cost. All this remains to be seen, however, as work to date has been performed only on prototypes, and a commercial product is thought to be 2 years away.



TRW, Inc.

Kwang-I Yu. *"This is not to say it is better than a supercomputer, but for this particular application, it has much more computing power."*

This unlikely marriage between TRW and Hood's group had its genesis some 3 years ago, when TRW's B. K. Richards heard a lecture at Stanford on the mathematics of genetics. The problem in DNA analysis, as Richards learned, is that the sequence consists of just four letters, the four nucleotide bases, repeated over and over again. How, then, do you extract the biologically meaningful information from the 3 billion letters that make up the human genome?

"Where are the 100,000 or so genes?" asks Hood. "What is the nature of the regulatory machinery? What are the sequences responsible for compactly folding in each and every cell 2 meters of DNA and 24 different chromosomes?" The answers are encoded in the string of letters.

To Richards, this decoding task seemed ideally suited to TRW's new chip, which was designed not to sift through DNA bases but to filter out important information in real time from the scads of cables and reports coming in to the Defense Department each day. A few weeks after the lecture, Richards met Nobel laureate Joshua Lederberg, president of Rockefeller University, who confirmed his suspicions. Richards, a Caltech alum, called the university and was put in touch with Tim Hunkapillar, a computer scientist in Hood's lab. Says Richards: "Tim came down to see the technology and in about 10 seconds said, 'This is a great idea.' "

Using the TRW chip and a Sun 3 computer, Hunkapillar designed a prototype DNA analysis system and wrote the necessary software, which will be available free from Hood's lab. To commercialize the prod-