# Human Trials of New Cancer Therapy Begin

*Agents that stimulate cancer cells to differentiate and therefore cease dividing have been identified and are beginning to be tested clinically*

CURRENT chemotherapeutic drugs act for the most part by killing cancer cells directly. Recently, however, researchers have begun clinical trials of several agents that act instead by changing the biological properties of cancer cells so that they lose one of their major characteristics, namely, the ability to divide continuously. Once this happens, the cells should eventually die.

According to presentations at a meeting* on "Cell Diffferentiation and Cancer," which was held the end of March on Key Biscayne, Florida, the results of the limited studies performed so far in human patients have been mixed—some agents have shown more promise than others. Nevertheless, the researchers are sufficiently encouraged by the early results to continue pursuing the investigations.

The new approach is based on demonstrations that cancer cells can differentiate to maturity and therefore reach a state of development at which division ceases. The findings run counter to the more popular view that malignant cells are locked irreversibly in an immature state that allows them to proliferate uncontrollably. Although cancer cells do have the general characteristics of immaturity, that condition is not necessarily permanent.

Even the cells of naturally occurring tumors differentiate, according to Bert Vogelstein of Johns Hopkins University School of Medicine and his colleagues. They have found that certain types of leukemia cells can develop into apparently normal granulocytes, white blood cells named for the prominent granules they contain. In addition, Vogelstein and Stanley Hamilton, who is also at Johns Hopkins, have recently shown that cells of the adenocarcinoma type of colon cancer can develop into the types of cells normally seen in the colon lining. "The view that cancer results from a block in differentiation is naïve at best," Vogelstein says.

Moreover, the discovery that a number of agents, both naturally occurring and synthetic, enhance tumor cell differentiation, at least in laboratory tests, has pointed the way to clinical trials. "The principle has been proven that you can unlock cancer cells, forcing them to differentiate and die," says Saul Gusberg of Mount Sinai School of Medicine in New York City, who organized the cell differentiation meeting. "It is time to test the agents clinically."

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One of the agents that has shown promise is Ara-C, a drug that is already used for chemotherapy and has differentiation-enhancing effects as well as direct cell toxicity. According to Donald Kufe of the Dana-Farber Cancer Institute in Boston, studies with cultured leukemia cells indicate that the differentiation effects occur at lower concentrations than direct cell killing. "When you are getting cytotoxicity, you are not getting differentiation," he notes.

Kufe and his colleagues have used low doses of the drug to treat 30 patients with "preleukemia," a condition in which the bone marrow cells fail to mature properly. Individuals who have the condition may develop a full-blown case of leukemia and are highly susceptible to infections. Six of the preleukemia patients treated by the Dana-Farber group had complete, but temporary, remissions and another 14 improved somewhat.

Other trials of low dose Ara-C have also indicated that the therapy has potential for treating preleukemia and leukemia. Data compiled by Bruce Chabner of the National Cancer Institute and cited by Kufe at the meeting show that about 45% of 237 patients with acute myelogenous leukemia responded to the therapy with complete or partial remissions. Approximately 35% of the preleukemia patients treated had complete or partial remissions. "It [low-dose Ara-C] has activity but the question remains as to what the mechanism is," Kufe says. Although there are some indications that it might be acting by inducing differentiation of the patient's cancer cells, as it does with the cultured leukemia cells, this has not been proved.

Other current chemotherapeutic drugs also have both cell killing and differentiation-enhancing effects, although determining which effect predominates may be difficult. As Alan Sartorelli of Yale University School of Medicine points out, "It is hard to distinguish the two actions in culture because the result is the same—cessation of cell growth."

Sartorelli and his colleagues have nevertheless been able to separate the two actions for the chemotherapeutic drug 6-thioguanine, an analogue of one of the bases that occurs in DNA. They did this with the aid of cells that lack the enzyme hypoxanthine phosphoribosyltransferase (HPRT), which converts bases such as 6-thioguanine to the nucleotide derivatives. According to Sartorelli, the 6-thioguanine nucleotides have the cell-killing activity, whereas the base itself enhances differentiation, an effect that becomes more evident in the HPRT-deficient cells because conversion of the base to the nucleotide is reduced.

The Yale workers have carried out a preliminary trial in which they attempted to treat acute leukemia patients with low doses of 6-thioguanine. Low doses were used in an attempt to avoid the cell-killing effects of higher ones. The patients did not respond to the treatment, however. Sartorelli speculates that high HPRT activity in the patients' cells may help to explain the treatment failure.

Strategies that might improve the results include giving another base, inosine, with the 6-thioguanine. Experiments with cultured cells have shown that 6-thioguanine's effects on differentiation are potentiated by inosine, apparently because it is converted to a compound that competes with 6-thioguanine for HPRT. Sartorelli also suggests that making analogues of 6-thioguanine that are not capable of being converted to nucleotides might be another approach that could be used to develop new differentiation therapies.

Another drug that is currently moving from laboratory to clinical studies is hexamethylene bisacetamide (HMBA), which is not currently used in in cancer chemotherapy. Paul Marks and his colleagues at Memo-

*The meeting, which was dedicated to Mary Lasker, a leading patroness of cancer research, was sponsored by the American Cancer Society and held on 30 March to 1 April.

rial Sloan-Kettering Cancer Center found that HMBA induces the differentiation of mouse erythroleukemia cells, which are derived from cells that differentiate into red blood cells. One indication of the compound's action is a marked stimulation of the expression of the genes coding for the globin proteins, which are needed for making hemoglobin, the oxygen-carrying pigment of red blood cells.

Richard Rifkind, also of Memorial Sloan-Kettering, described the results of a preliminary clinical trial of HMBA in patients with advanced leukemia. The goal of the trial was to assess how best to administer the drug and how well its side effects are tolerated. HMBA's side effects include decreased numbers of blood platelets, which are needed for blood clotting, and increased acidity of the blood, which contributed to a depression of the brain functions. The Sloan-Kettering workers are currently trying to redesign the drug to avoid these side effects without diminishing HMBA's effects on cell differentiation.

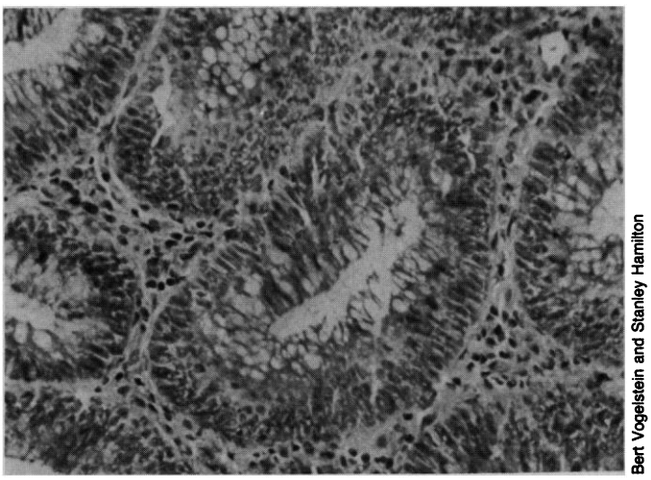
Researchers are also beginning clinical tests of naturally occurring agents that elicit cell differentiation in the body. The most prominent of these are the hematopoietic growth factors, which are necessary for the production of the red blood cells and various types of white blood cells. Malcolm Moore of Memorial Sloan-Kettering Cancer Center in New York City described his group's work with granulocyte–colony-stimulating factor (G-CSF), which induces the production and activity of the neutrophils, a type of granulocyte that is important in fighting bacterial infections.

According to Moore, G-CSF causes myeloid leukemia cells that are grown in culture to differentiate to neutrophils and to lose their potential to divide. The agent also greatly extends the life of leukemic mice. Untreated animals die within 60 days of the onset of symptoms. But 80% of those given G-CSF survived for at least 6 months, at which time the study was discontinued. Moreover, the agent only had to be administered for 28 days. "You don't need to continue treatment after the replicating cells are gone," Moore explains. This is an encouraging finding because it indicates that the treatment does not have to be continued indefinitely.

In addition to the possible direct use of G-CSF to fight cancer, it and the other hematopoietic growth factors may also be used to help protect patients against one of the major side effects of drug and radiation therapy. Many of these treatments suppress the bone marrow and cause consequent decreases in blood cell production, thereby leaving the patients open to infections and bleeding problems. Moore and his colleagues have found that G-CSF counteracts the neutrophil deficiencies caused in mice by the chemotherapeutic drugs Cytoxan and 5-fluorouracil.

**Differentiation in cancer cells.**

*The colon cancer (adenocarcinoma) shown here contains differentiated cells similar to those of the normal colon lining. They form gland-like structures (with the oval shapes) and can secrete mucus.*



Bert Vogelstein and Stanley Hamilton

The addition of interleukin-1 to the treatment may potentiate G-CSF's effects in protecting mice against the side effects of 5-fluorouracil, according to Moore. Interleukin-1 is another of the agents that is needed for normal differentiation and function of white blood cells. Its effects include increasing the number of the cell surface receptors to which G-CSF and certain of the other hematopoietic growth factors must bind before they can influence cellular activities.

Not all of the drugs that have shown promise in laboratory or animal tests have proved successful in clinical trials, however. For example, H. Phillip Koeffler of the University of California School of Medicine in Los Angeles reported the results of trials with two agents, 13-*cis*-retinoic acid and a vitamin D derivative. Although both compounds induce the differentiation of leukemia cells in laboratory cultures, neither produced detectable improvement in the blood counts of preleukemia patients, according to Koeffler.

A better understanding of the forces guiding normal and cancer cell differentiation might enable the design of better strategies for eliciting cancer cell differentiation. For example, one of the earliest indications of the ability of cancer cells to develop normally came a few years ago when researchers showed that cancer cells lose their tumor-forming potential and even contribute to normal tissue formation when placed in an embryonic environment. Recently, G. Barry Pierce and his colleagues of the University of Colorado Health Sciences Center in Denver have identified a soluble factor in fluid from early mouse embryos that inhibits the division of embryonal carcinoma cells, which are derived from the germ cell tumor known as a teratoma.

The Pierce group had previously shown that the embryos suppress the tumor-forming potential of the carcinoma cells, and the factor may contribute to that suppression. It does not work alone, however. The Colorado workers have found that contact of the the tumor cells with certain of the embryonic cells is also required. Nevertheless, if the embryonic substances that elicit cancer cell differentiation can be isolated, they might have potential use in the clinic.

At least one of the differentiation-enhancing drugs may also work by eliciting the synthesis of "differentiation factors." Michio Oishi of the University of Tokyo described his group's identification of two factors that are produced by murine erythroleukemia cells and work cooperatively in effecting the differentiation of the cells. One of the factors is induced by drugs such as HMBA and works only in the erythroleukemia cells. The second is elicited by agents, such as ultraviolet light, that damage the cellular DNA; its action is not limited to a specific cell type. The Tokyo workers are now trying to purify and characterize the factors.

Finally, the question might arise as to how malignant tumors form in the first place if cancer cells are so capable of differentiation. In a normal adult tissue that is not growing, cells that die are replaced by the division of the immature cells known as stem cells. This division ordinarily produces equal numbers of stem cells and cells that differentiate to maturity.

But cancer could result, Pierce and Vogelstein hypothesize, if some alteration shifts the balance in favor of greater stem cell production. "Malignant stem cells can produce terminally differentiated progeny, but make many more malignant cells," Pierce explains. "Tumors are caricatures of the process of tissue renewal." The new work on tumor differentiation aims to shift the balance back again, thereby removing the potential for uncontrolled growth from the tumor cells. ■ JEAN L. MARX