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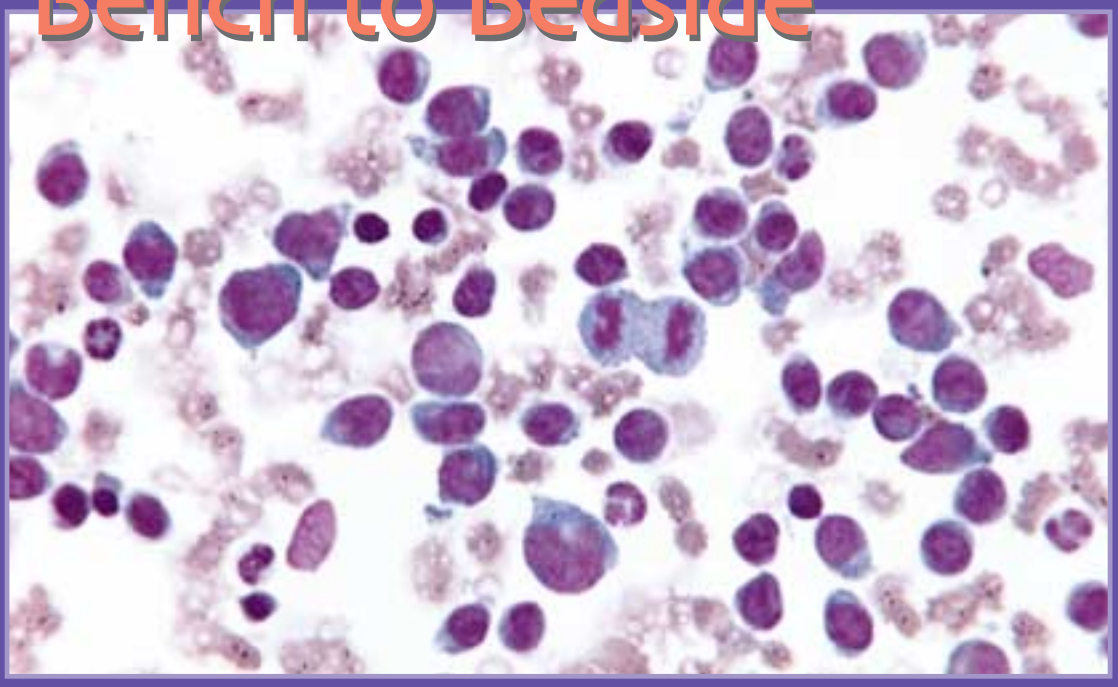
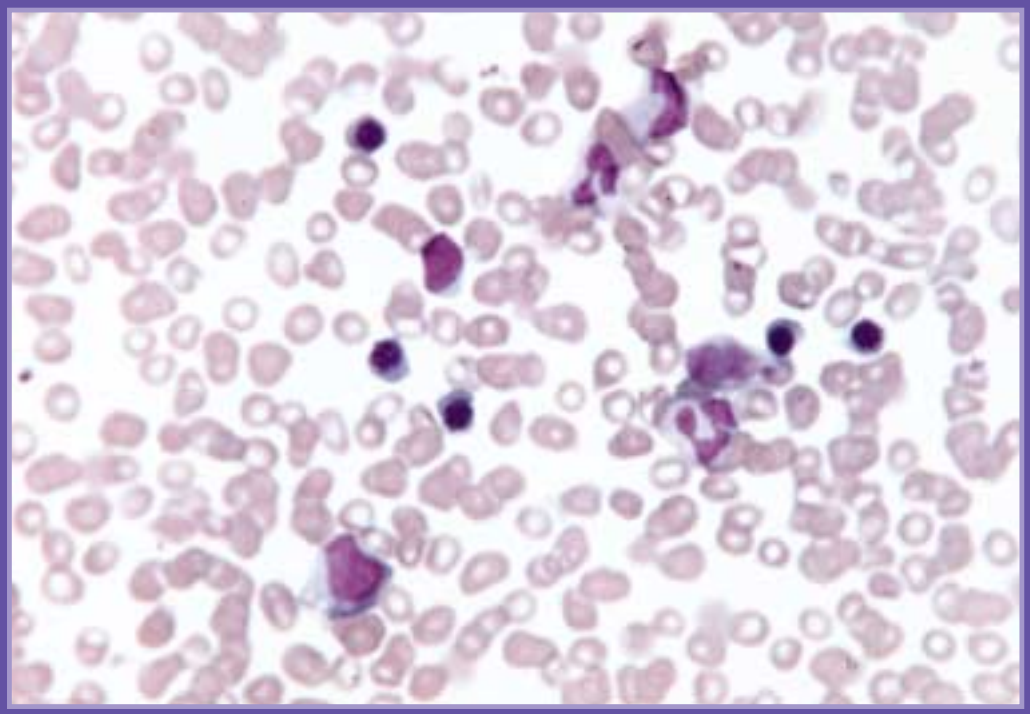
Targeting Leukemia: From Bench to Bedside





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TARGETING LEUKEMIA-FROM BENCH TO BEDSIDE

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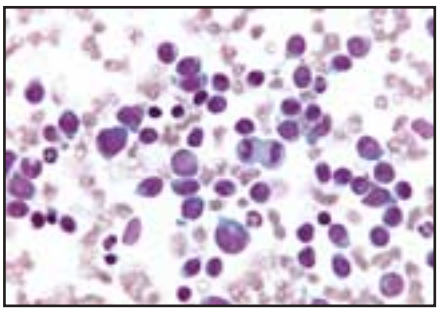
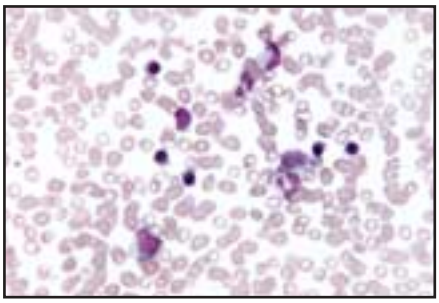


PANEL A



PANEL B

COVER IMAGE: Panel A is a bone marrow sample from a patient with acute leukemia. The dense, immature leukemic cells outnumber the normal white blood cells, which are smaller. A leukemic cell in the center of the field is in the process of dividing, as reflected by the presence of two sets of chromosomes seen in the nucleus of the dividing cell. Panel B is the bone marrow sample from a leukemia patient in total remission following therapy. The cells present are all normal bone marrow cells and they show the diversity in cellular structure that reflects the mixture of cell types present in normal bone marrow. Image @ 2001, Richard G. Lynch, M.D., University of Iowa.

TARGETING LEUKEMIA: FROM BENCH TO BEDSIDE

"It is that bridge between the clinic and the laboratory that sustains the ability to control disease."

—Emil Freireich
Leukemia (1998) 12, Suppl. 1, S56

In the 1960's, many doctors were drafted into the military unit of the Public Health Service. Some of these men ended up working on the children's leukemia ward of the National Cancer Institute's Clinical Center. The men never encountered the jungles of Viet Nam and the horrors of war. But each day, after passing the well-kept grounds of the Clinical Center and entering the newly built brick building, they encountered a different sort of horrendous scene.

The children's leukemia ward was filled with the chatter of little ones, some as young as two years old, who, clutching their teddy bears, toy cars or dolls, couldn't expect to live more than a matter of months. One child made a particularly indelible impression on her doctor. "I really want to finish the fourth grade," she confided in him. "Do you think I can?"

Four decades later, nearly 80 percent of all children with leukemia survive the disease—twice the survival rate in 1970. Impressive survival gains have also been made for adults with this type of cancer. Surprisingly, many of the important strides made in the diagnosis and treatment of leukemia did not hinge on the efforts of cancer researchers who specifically aimed to conquer the disease, but rather by a diverse bunch of curious scientists from pathologists, hematologists and immunologists, to nutritionists, chemists and geneticists. These researchers, who, by pursuing the answers to such basic questions as "why do liver extracts cure anemia?", and "how can we detect life on Mars?", collectively homed in on the molecular defects that underlie leukemia and ways to counter those flaws.

White blood

The success story of leukemia is actually a medical detective story that got its start in 1845, when Rudolph Virchow, a young Berlin pathologist, was puzzled by the strange set of symptoms displayed by one of his patients. The 50-year-old cook had been admitted to the hospital complaining of fatigue, frequent nosebleeds and swelling of the legs and abdomen. She died just four months later.

Following an autopsy, Virchow slipped a slide of the patient's blood under a microscope. Peering into the eyepiece, he was amazed to discover that the patient had a lack of red blood cells and an excess of white or colorless cells. Virchow coined the term "leukemia", or "white blood," to describe the patient's condition. He, along with the Scottish pathologist John Hughes Bennett, who independently had made similar discoveries at the same time, began a quest to understand what exactly goes wrong in this unusual disease.

A clue that helped solve that mystery came about ten years later when another pathologist, Ernst Neumann, was conducting an autopsy on a patient who died of leukemia. He discovered that the bone marrow wasn't its normal red color, but rather had become "dirty green-yellow." This, among other findings, led him to conclude that something awry in the bone marrow was responsible for the abnormal blood of leukemia patients. It also led him to discover that the bone marrow was an important site for blood cell formation.

But many of the mysteries of blood formation and leukemia were not tackled until the innova-

tive use of cell stains at the end of the 19th century. These stains enabled researchers to make finer distinctions between the cells they saw under a microscope. This laboratory research led to the surprising discovery that blood cells, like the animals that produce them, do not start out fully mature and able to conduct all the tasks performed by older cells. Instead "ancestor" cells give rise to two types of primitive white blood cells known as myeloblasts and lymphoblasts. Over time, lymphoblasts develop into the T or B cells that are known for fighting infections. Myeloblasts eventually develop into a number of cell types including those that fight infections, foster allergic reactions, or help the blood clot.

Leukemia occurs when a white blood cell, whose development is frozen, continues to duplicate itself. The resulting progeny of cells are all in the same stage of development and bear the distinctive hallmarks of the type of ancestral white blood cell that gave rise to them.

Based on this understanding, by 1900 leukemia was no longer seen as a single disease. Instead it was imagined akin to a tree with two main limbs that in turn have two primary branches, all of which reflect from what type of cell the leukemia originates. (See Figure 1). One limb, myelogenous leukemia, has as its hallmark in the blood and bone marrow either a predominance of immature myeloblasts (one branch termed acute myeloid leukemia) or mature myeloid cells (other branch termed chronic myeloid leukemia). With the other limb, lymphocytic leukemia, the blood and bone marrow is overpopulated by either precursor B or T cells (one branch called acute lymphocytic leukemia) or mature B or T cells (other branch called chronic lymphocytic leukemia).

Despite their divergent origins, all types of leukemias share the same set of symptoms, just as fever and congestion can be the symptoms for a number of different microbial infections. When people develop leukemia, their abnormal white blood cells crowd out or hamper the functioning of their red blood cells, fostering the tiredness and paleness that are the hallmarks of anemia. These white blood cells also do not effectively fight infections, which can be fatal. A lack of functioning cells (platelets) that clot blood makes people with leukemia prone to life-threatening bleeding episodes. Leukemia cells that congregate at various spots in the body can also spur a variety of other symptoms, including bone or joint pain, or enlarged organs. Untreated acute leukemia progresses rapidly to death, whereas untreated chronic leukemia can be longer lasting, although just as deadly in the long run.

Although leukemia afflicts both adults and children, by the 1920's it was recognized that the disease mostly attacks children. At about this time, leukemia began an alarming rise in incidence. In 1930 it was considered a relatively rare disease. But by 1960, statistics collected in Great Britain revealed that leukemia had become the second leading cause of death in children.

This rising incidence of leukemia in children reflects the explosion of knowledge about infectious diseases that began

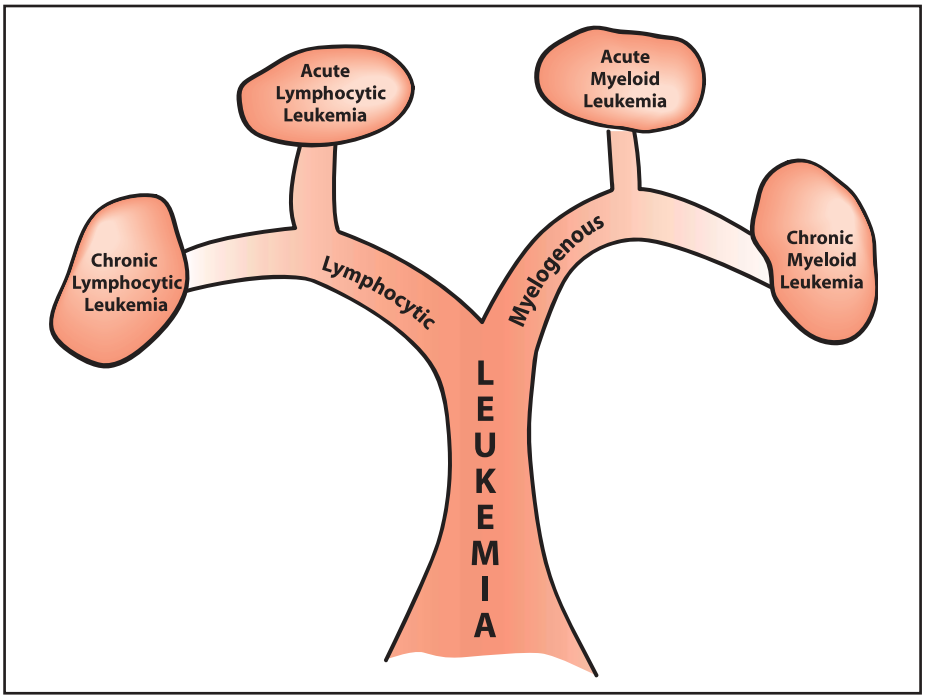


Figure 1. LEUKEMIA TREE By 1900, leukemia was no longer seen as a single disease, but rather akin to a tree with two main limbs that in turn have two primary branches, all of which reflect from what type of blood cell the leukemia originates.

with the pioneering work of Robert Koch and Louis Pasteur. At the end of the 19th century, these scientists started the search for the microbial causes of many common contagious diseases. By the middle of the 20th century, researchers had pinned down the culprits responsible for many of the infections that were life threatening to children, including diphtheria, pneumonia, and tuberculosis. Their findings fostered the development of antibiotics and vaccines to cure or prevent many of these ills, allowing leukemia to come to the forefront as a major cause of death among children.

Leukemia's emerging importance, however, was not accompanied by an improved understanding of what caused the disease or how it could be treated. Indeed, when leukemia first became more common, researchers were still debating whether it was an infectious disease or a cancer. This lack of understanding of leukemia hampered the development of effective drugs for the disease, which quickly wreaked its tragic effects. In 1936, children lived only a few weeks after being diagnosed with leukemia.

Dreaming of a cure

But hope was on the horizon, thanks to a Harvard doctor who was curious as to what it was in liver extracts that cured his patients of anemia that was not caused by leukemia. He tested the newly discovered vitamin, folic acid, which is abundant in liver, and found it fostered the production of red blood cells in his patients. Following his lead, a number of doctors started to use folic acid to treat the anemia their leukemia patients experienced. But Sydney Farber, a Boston pathologist, noticed that patients worsened when they were given the vitamin. This gave Farber the idea that if folic acid accelerated the progress of leukemia, maybe the disease could be slowed down by a compound that counters folic acid in the body.

A persuasive man, Farber convinced chemists at Lederle Laboratories to concoct a compound that would block the action of folic acid. This was possible because laboratory researchers had recently elucidated the structure of the vitamin. Using this information, scientists at a pharmaceutical company created an inactive folic acid mimic that, by substituting for the real thing, prevented folic acid from substantially affecting blood cells.

In 1947, despite the objections of many, who thought children with leukemia should be left to die in peace, Farber and his colleagues began treating a small number of children with acute lymphocytic leukemia (ALL) with the folic acid mimic, called aminopterin. The researchers were encouraged when a majority improved, including one child who showed no signs of the disease for several months. Referring to this child, Farber's colleague Robert Mercer reflected in a recent issue of the scientific journal *Medical Pediatric Oncology*, "The bone marrow looked so normal that one could dream of a cure."

Unfortunately, none of the patients given aminopterin were cured of their leukemia. But the limited success of these researchers sparked others to pursue a search for drugs that could stymie the progress of leukemia.

Discovery of the next leukemia drug stemmed from the laboratory findings that cells need DNA to divide, and that one of the major building blocks of DNA are compounds called purines. Building on that information, chemists George Hitchings and Gertrude Ellion of Wellcome Research Laboratories concocted a purine look-alike called 6-mercaptopurine (6-MP). Like a misshaped object in a factory assembly line, this compound jammed DNA production and stopped leukemia cells from dividing.

Other important early anti-leukemia drugs were discovered when laboratory researchers were pursuing seemingly unrelated avenues. For example, testing on rats the effects of a folk remedy for diabetes—an extract of the periwinkle plant—led to the discovery of vincristine. Other research aimed at seeing if extracts from adrenal glands could mimic the beneficial effects of pregnancy on rheumatoid arthritis led to the discovery of another drug called prednisone. These compounds are mainstays in many anti-cancer drug regimes.

But none of these drugs, singly, were able to save children with acute leukemia despite their teasing ability to temporarily erase any obvious signs of the disease. What made the leukemia come

back? This was not only an intriguing puzzle, but also a life-threatening dilemma to the children afflicted with the disease.

It took an innovative and diverse team of clinicians, pharmacologists, and geneticists to make any headway into solving that puzzle. One member of the team, geneticist Lloyd Law of the National Cancer Institute (NCI), developed a way to study leukemia in mice that proved immensely valuable in ascertaining why the remissions were so fleeting. Another team member was pharmacologist Howard Skipper, then at the Southern Research Institute, who used to say, "a model is a lie that helps you see the truth."

Skipper applied pharmacology principles and mathematical modeling techniques to findings from Law's leukemic mice to show that although the new cancer drugs might kill nearly all leukemia cells so that no residual disease could be detected via standard means, a small number of leukemia cells was left lurking behind. These cancer cells could eventually multiply to the point of causing a resurgence of the disease. This suggested that if doctors wanted to cure their patients of leukemia, they had to continue to treat them even after their symptoms disappeared.

Another key piece to the puzzle came when Law discovered that when he treated his leukemic mice with 6-MP, their leukemia cells eventually stopped responding to the drug. But a glimmer of hope came from the additional finding that although these leukemia tumors developed resistance to 6-MP, they became more sensitive to the folic acid mimic methotrexate. This drug readily killed the leukemia cells.

Law's intriguing findings suggested to NCI hematologists and

Rudolph L. K. Virchow

Christian Neumann

Sydney Farber

Howard Skipper

Emil J. Fredriech, Jr.

Emil Frei III

TIMELINE

1845- Pathologists Rudolph L. K. Virchow and John Hughes Bennett first describe leukemia as a medical disorder with distinctive hallmarks in the blood.

1850s- Pathologist Ernst Christian Neumann discovers that the bone marrow is abnormal in leukemia patients.

1900- Innovative cell stains reveal that leukemia is not a single disease, but a collection of at least four different types.

1940s- Pathologist Sydney Farber discovers that an inactive folic acid mimic (aminopterin) can transiently improve some children with leukemia.

1950s and 1960s- Basic research leads to the discovery of several anti-leukemia drugs, including 6-mercaptopurine, vincristine, and prednisone.

1950s and 1960s- Geneticist Lloyd Law and pharmacologist Howard Skipper discover why leukemia recurs when patients are treated with just one drug.

1962- The first leukemia cures are achieved when Emil J. Freireich, Jr. and Emil Frei III begin treating patients with combination chemotherapy.

1960- Pathologist Peter Nowell and David Hungerford discover that in the cancerous white blood cells of several patients with chronic myeloid leukemia, a portion of one of their chromosomes is missing.

Figure 2.

clinical researchers Emil J. Freireich Jr., and Emil Frei III that they might get the best results if they treated patients with all four anti-leukemia drugs currently available: vincristine, amethopterin, 6-MP, and prednisone. They began treating patients with that regimen in 1962. Many of these patients are still alive today—living testimony to Farber's audacious decision to treat patients with leukemia instead of merely allowing them to die.

However, another major stumbling block remained. The majority of patients experiencing complete and long-lasting remissions eventually developed an excess of leukemia in their brains that killed them. Going back to the lab bench, researchers discovered why—the drugs that were so effective at killing leukemia cells weren't penetrating into the spinal cord or brain. Leukemia cells flourished in these central nervous system hideouts, eventually causing fatal complications. Fortunately, animal studies showed a way out of this predicament—direct injection of the cancer drugs into the spinal-cord canal and irradiation of the head (brain).

Additional drugs and improvements in supportive therapy also helped bring the cure rate of childhood ALL up to its present high rates. But even when radiation and drug therapy cures children of leukemia, the treatment is so toxic that it can cause a number of serious complications, including the development of a new type of leukemia or brain tumors or heart problems. Part of the toxic nature of the therapy resides in the fact that it is not specific—it doesn't affect just leukemia cells, but any actively dividing cell. Not all types of leukemia, in addition, are cured by the regimen that is usually


Peter Nowell


Leonard Herzenberg


Janet Rowley


Kary Mullis


Brian Druker

1970-
The first flow cytometer is developed by immunologist Leonard Herzenberg and his colleagues.

1973-
Hematologist Janet Rowley discovers that chronic myeloid leukemia gets triggered by a piece of chromosome 22 breaking off and reattaching onto chromosome 9 in a white blood cell.

1980s-
Researchers discover the fused gene (BCR-ABL) that is created when a portion of chromosome 22 translocates to chromosome 9.

1980s and 1990s-
The use of flow cytometry reveals more than a dozen different subtypes of leukemia and aids the detection of leukemia cells surviving treatment.

1985-
Biochemist Kary Mullis first conceives of polymerase chain reaction (PCR).

1990s-
PCR is used to fine-tune leukemia diagnosis and detect leukemia cells that survive treatment.

1990s-
Oncologist Brian Druker discovers how BCR-ABL triggers excessive division of white blood cells and shows that STI571, a compound developed by Novartis, has anti-BCR-ABL activity in cell cultures.

2001-
After human testing reveals STI571's effectiveness, the Food and Drug Administration approves it under the name Gleevec, as a drug for chronic myeloid leukemia patients.

effective for childhood ALL. For example, the chemotherapy regimen for ALL is not usually effective for chronic myeloid leukemia (CML).

Genetic clues

To develop more targeted and non-toxic treatments for various types of leukemia, scientists needed to delve deeper into the conundrum of what exactly causes them. An important clue that guided this journey was that exposure to high amounts of radiation, such as that emitted by the atomic bomb dropped in Hiroshima, damaged the gene-harboring chromosomes of survivors and made them prone to developing leukemia. This suggested that the root of a cancer cell's wayward behavior lies in the genetic machinery of the cell.

A University of Pennsylvania pathologist Peter Nowell provided a view into that genetic machinery, in 1960. He was trying to discern chromosomal abnormalities in leukemic white blood cells. To rid blood specimens of red blood cells so he could study the remaining white blood cells, Nowell treated the specimens with a plant compound called phytohemaglutinin. This chemical caused the red blood cells to clump together for easy removal.

One day, when he went to collect the blood of a leukemia patient for his research, he discovered the patient had gone into remission. But when Nowell examined the patient's white blood cells he was surprised to see evidence that they were dividing. At the time, it was thought that normal white blood cells in the blood were incapable of dividing, unlike cancerous white blood cells. So Nowell decided to see how these patients' cells compared to the normal white blood cells found in his own blood.

When Nowell peered under the microscope to examine his white blood cells, he was surprised to discover stretched across their nuclei in an orderly fashion, 23 pairs of worm-like structures he immediately recognized as chromosomes. When a cell divides, its chromosomes must disentangle themselves from their usual incomprehensible nuclear jumble and line up in the duplicate pairs that Nowell saw in his own cells. By essentially catching them in the act of reproducing, Nowell had a detailed view of human chromosome structures in normal blood cells.

After a process of elimination, Nowell discovered that the plant compound he used to sift out his red blood cells had stimulated his white blood cells to divide. This compound opened the door to the easy study of how the chromosomes of leukemic white blood cells compare to those of normal white blood cells. In 1960, Nowell and his colleague David Hungerford reported that in the cancerous white blood cells of several patients with CML, a portion of one of their chromosomes was missing.

Where had it gone?

This was a mystery until a dozen years later when innovative stains fostered unique striping patterns that researchers could use to identify each chromosome. Using these stains, cytogeneticist Janet Rowley of the University of Chicago noticed that in the white blood cells of patients with CML, chromosome 9 was longer than normal and the additional length had a striping pattern just like that of the portion of chromosome 22 that was missing in these patients.

Rowley had found the smoking gun—the cutting and pasting of a piece of chromosome 22 onto chromosome 9 apparently triggered these patients' leukemias. But why was this "translocation" a problem?

It took nearly two decades and the basic research of several laboratories to answer that question. The winding DNA that comprise chromosomes have sections (genes) that direct the production

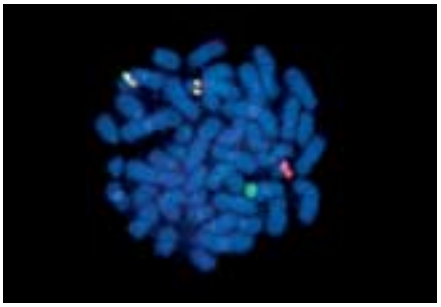


Figure 3. An image of a dividing cell from a patient with chronic myeloid leukemia (CML). CML is triggered upon the cutting and pasting of a piece of chromosome 22 onto chromosome 9, resulting in a protein called BCR-ABL. The above cell shows the BCR probe labeled in green on the normal chromosome nine and the ABL probe labeled in red on the normal chromosome 22. The abnormal chromosomes 9 and 22 are labeled with a fusion signal that is green and red with a yellow area of overlap. Image @ 2001, Janet D. Rowley, University of Chicago.

of specific proteins. But much of the chromosomes are comprised of barren stretches of DNA that apparently lack genes. However, research revealed that when the translocation that Rowley detected occurs, chromosome 22 breaks off right in the middle of a length of DNA that is a gene. To top it off, this chunk of chromosome lands smack in the middle of another gene on chromosome 9. The fused genes produce a new protein called BCR-ABL. This protein quickly rose to fame as a major suspect in the cause of CML.

In the 1980's, a research team led by the molecular biologist David Baltimore, then at the Whitehead Institute for Biomedical Research in Cambridge, set out to learn more about this suspect. His lab's research indicated the BCR-ABL protein was a kind of enzyme called a tyrosine kinase.

This discovery set off bells in biochemistry labs because laboratory research has revealed that tyrosine kinases play a key role in governing the proper growth and division of cells. It is precisely these processes that go awry in leukemia and other cancers. Nearly 50 tyrosine kinases have been implicated as playing key roles in the development of various types of human cancers, including leukemia, breast and bladder cancer. Consequently, tyrosine kinases have become hot new targets for anticancer drugs. (See sidebar, *Tyrosine Kinases and Cancer,* on page 9).

One researcher prompted to pursue the tyrosine kinase trail was Brian Druker, an oncologist

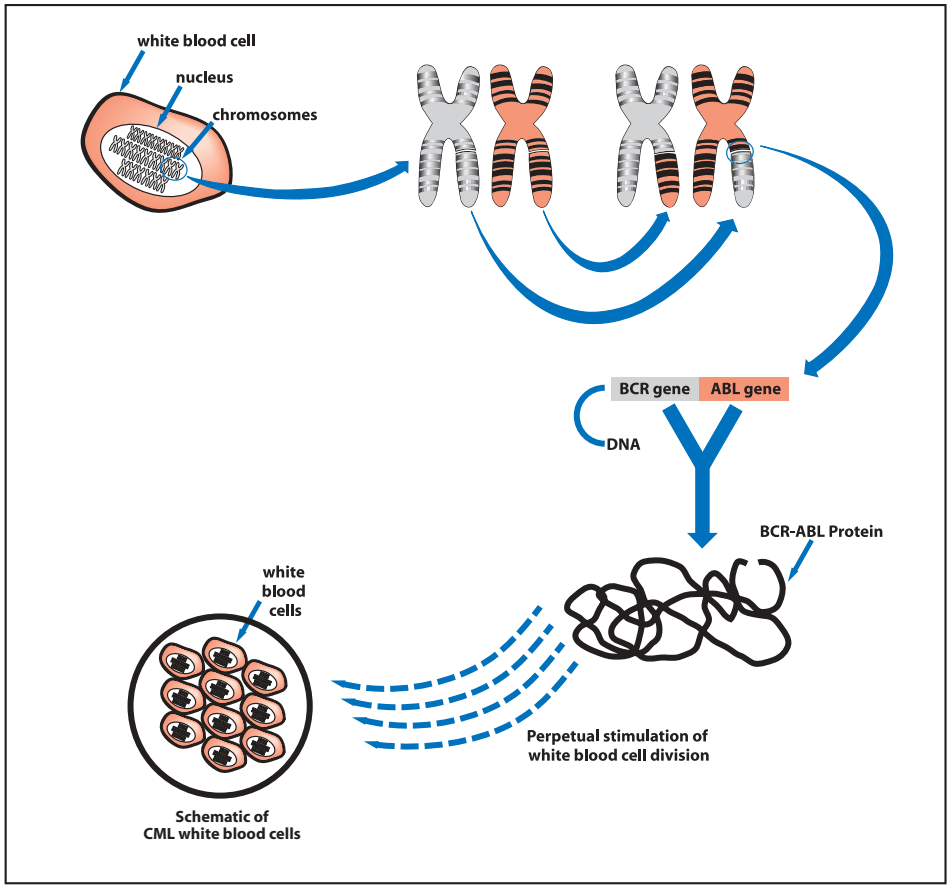


Figure 4. SCHEMATIC OF DEVELOPMENT OF CML
Chronic myeloid leukemia is triggered when within a white blood cell a portion of chromosome 22 breaks off and reattaches to chromosome 9. This causes the BCR gene from chromosome 22 to attach to the ABL gene of chromosome 9. The BCR-ABL fused gene directs the production of a protein that prompts the white blood cell and all its progeny to divide excessively. *Designed by Corporate Press.*

who became fed up with the state-of-the-art of cancer care. About the time that Baltimore pegged BCR-ABL as a tyrosine kinase, Drucker decided to venture into the lab at the Dana-Farber Cancer Institute in Boston to gain a better understanding of how exactly BCR-ABL might trigger leukemia.

Laboratory research has revealed that tyrosine kinases usually trigger cell growth or division by activating key molecules known as signaling proteins. The kinases prompt these signaling molecules into action by decorating them with phosphates that they remove from ubiquitous molecules in cells called ATPs. This process is known as phosphorylation.

What did BRC-ABL phosphorylate? Druker recognized that the answer to this question would be key to understanding how the fused genes cause leukemia. So he compared the phosphorylated compounds generated by blood cells that had the BCR-ABL fusion gene inserted, to the phosphorylated compounds made by blood cells that lacked the gene.

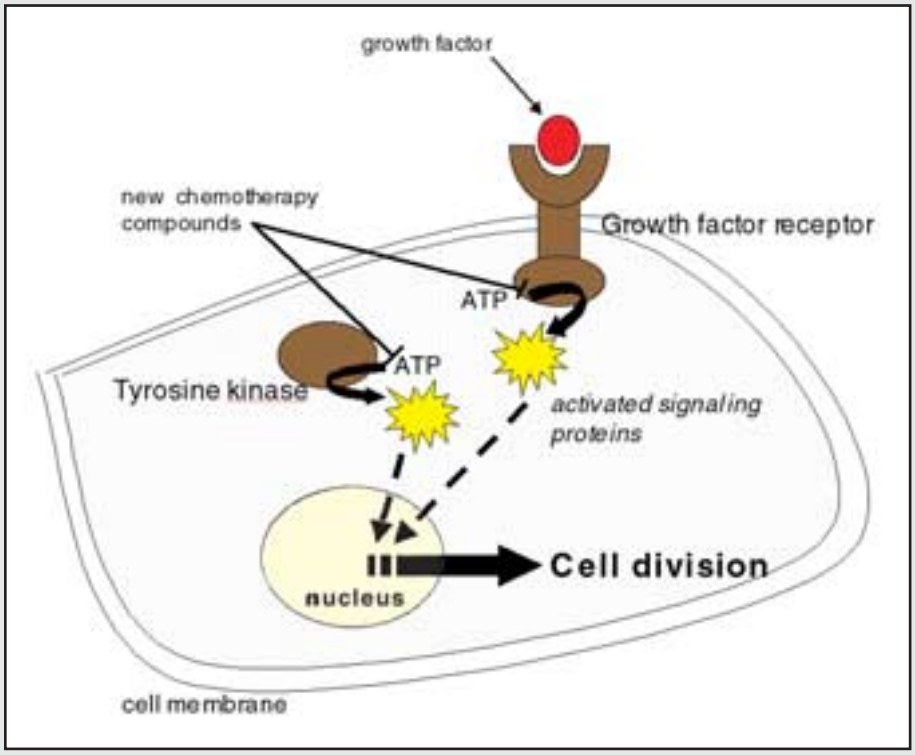
This led Drucker to discover that the BCR-ABL protein activates a molecule (rasGTPase activating protein) that plays a key role in prompting cells to divide.

Unlike normal tyrosine kinases, however, BCR-ABL lacks a vital section that enables it to eventually shut off and stop the chain of events that results in cell division. Like a stuck throttle, BCR-ABL revs up certain white blood cells, prompting them to continually divide. The end result is CML.

With this information in hand, Druker teamed up with researchers at Ciba-Geigy, now known as Novartis. These scientists had concocted several compounds designed to inhibit the activity of various tyrosine kinases. They handed these compounds over to Druker, who tested their ability to inhibit the activity of BCR-ABL in blood cells that had the fused gene. One of these compounds, called STI-571, showed promise.

In 1998, Druker and his colleagues began testing STI-571 on CML patients who weren't responding to the immune stimulant interferon, which was the gold standard of therapy for this type of leukemia even though it has many serious side effects and fails to cure the majority of patients. Three years later, the researchers were amazed to report that STI-571 had proved safe and effective for an amazing 53 of 54 patients who received it in high doses. Some patients, who were practically planning their funerals at the beginning of the study, felt remarkably better after taking the drug for just a few months. Equally impressive is the fact that no serious side effects of STI-571 have been reported yet.

Tyrosine Kinases and Cancer



Although there are more than a hundred different kinds of cancers, each with their own unique hallmarks, all cancers can be characterized by an excessive amount of cell proliferation. Indeed, cancer cells become deaf to the normal controls on cell growth and continually divide.

Insight into what causes that lack of control has come from laboratory research on normal cell division and what steps in that process are corrupted in cancer cells. Compounds known as growth factors are manufactured in the body to stimulate cell division and cell growth. These compounds bind to antenna-like growth factor receptors lodged in cell membranes. When a growth factor attaches to its receptor, it triggers a cascade of chemical signals inside the cell that carry the message that cell division is needed to the cell nucleus, which starts the cell splitting process. Key molecules in this signaling pathway are tyrosine kinases.

These enzymes often act as chemical messengers between growth factor receptors and other signaling proteins that stimulate cell division. To prompt these signaling molecules into action, tyrosine kinases transfer phosphate groups from ATP, a molecule that is abundant in all living cells, onto specific sites on the signaling molecules. These activated proteins in turn prompt cell growth and division.

The actions of tyrosine kinases in normal cells are closely controlled by growth factors and other enzymes called tyrosine phosphatases, which remove the phosphates and act as brakes to arrest growth and division. But in cancer cells, the tyrosine kinases are often overproduced or made in forms that are active all the time and no longer dependent on growth factors. Some cancer cells also lack functional tyrosine phosphatases. Because they lack these brakes on cell growth and division, cancer cells continually divide resulting in tumors, or in the case of leukemia, excessive amounts of certain kinds of white blood cells in the bloodstream and bone marrow. To counter that excessive cell division, many new chemotherapy compounds target the actions of tyrosine kinases.

For more information, refer to Jensen-Blume, P. & Hunter, T. 2001. Oncogenic kinase signalling. Nature; 411:355-365.

It's too early to tell if STI-571, which goes by the trade name Gleevec, can improve the long-term survival of CML patients. But the remarkable results so far prompted the Food and Drug Administration to approve it for use in these patients.

Gleevec is just one of several promising treatments for leukemia that have recently been discovered because laboratory research has uncovered the cause of a specific leukemia. For example, Rowley discovered that the leukemia trigger for patients with acute promyelocytic leukemia (APL) is a translocation between their chromosomes fifteen and seventeen. This chromosome swapping disrupts the normal functioning of a receptor for retinoic acid, a form of vitamin A. It is thought that because the receptor does not work properly, the cells are essentially starved for retinoic acid. This compound stimulates the maturation of certain cells and, when it is given to APL patients, it enables their leukemia cells to develop normally, thereby boosting long-term survival.

However, findings in some patients suggest that the effectiveness of both retinoic acid and Gleevec may be hampered by the development of drug resistance. Thus, these drugs may have to be combined with others to generate cures for these leukemias.

But many agree that this strategy for combating leukemia holds great promise. In the two decades since Rowley and her colleagues learned that a specific chromosomal translocation gives rise to a specific type of leukemia, she and other researchers have pinpointed over a hundred different translocations in the cells of various leukemia patients. Scientists are beginning to decipher which of these translocations are vital in causing leukemia. The presence of various common translocations is already being used to classify patients' leukemias. This classification scheme has proven extremely valuable in deciphering why two patients, whose leukemias fall under the same broad category of leukemia (ALL, for example), can have such different responses to treatment. It has also led to more tailored treatments, as some drugs work better on leukemia cells with certain kinds of translocations.

The ability to make fine distinctions in leukemia types is revolutionizing the treatment of patients with these cancers. This is possible not only because of the advances made in uncovering the genetic causes of leukemias, but also stems from the work of researchers from an unusual potpourri of disciplines from immunology to physics. These scientists weren't bent on finding a cure for cancer, but rather had a diverse bunch of objectives, including developing equipment to detect extraterrestrial life and trying to find the genetic basis for antibody diversity.

Go with the flow

A device that has proven immensely valuable in leukemia diagnosis and treatment, the flow cytometer, was dreamed up by space scientists who wanted an automatic way to sift out extraterrestrial life from materials collected from Mars. To meet that task, the National Aeronautics and Space Administration (NASA) funded the research of a multidisciplinary team of scientists at Stanford University. But as support for the research team shifted from NASA to NIH, the research emphasis shifted from designing a device useful to space exploration to developing one that would impact the medical arena—specifically a device that could help diagnose the rejection of transplanted organs by the immune system.

By 1970, the researchers led by immunologist Leonard Herzenberg debuted their prototype flow cytometer. This device had the amazing ability to channel a stream of a mixed population of cells so that the cells tumbled single file past a laser light. The cells were sorted based on how they scattered the laser light and by how much of a fluorescent dye they absorbed and emitted. The flow cytometer not only separated the various cell types for further study, but also counted how many of each cell type was in a sample. This device could sort cells at the remarkable rate of 5000 cells per second!

The researchers further refined their cell sorter by attaching antibodies to a fluorescent dye. These antibodies selectively bound to specific cell types, boosting their degree of fluorescence. But the researchers had trouble standardizing the original crude antibody mixtures they used because these mixtures reacted to more than one cell type and varied from batch to batch. This lack of standardization was limiting the usefulness of the flow cytometer.

Fortunately, a pair of immunologists trying to uncover the genetic basis of antibody diversity came to the rescue. For their laboratory research, Cesar Milstein and Georges Kohler, working in Cambridge, England, needed antibody-forming cells that could survive and perpetuate themselves for a long time in the laboratory cultures used for their experiments. So they fused antibody-forming cells with tumor cells, which are capable of maintaining themselves for long periods outside the body. The end result was a hybrid cell that not only was long-lived in the laboratory, but also produced only one type of (or "monoclonal") antibody.

In 1976, Herzenberg spent several months in Milstein's laboratory and immediately realized that these novel monoclonal antibodies were the solution to the problem he had standardizing his flow cytometer. He began using the monoclonal antibodies attached to fluorescent dyes in his flow cytometer several months later.

Researchers then used Herzenberg's refinements on flow cytometry to discern the various types of T and B cells. The surface of each of these types, as well as those of other white blood cells are studded with a distinctive array of proteins known as antigens. These telltale landmarks are invisible in stained cells viewed under the microscope. But they can be easily spotted with a panel of different fluorescent monoclonal antibodies. Each of these antibodies is designed to latch onto a specific cell-defining antigen and send out a red, orange or green glow that can be detected by the flow cytometer.

Thanks to the powerful combination of monoclonal antibodies and flow cytometry, researchers were able to refine the four basic categories of leukemia into more than a dozen different subtypes. These subtypes are classified according to the pattern of proteins they display on their cell surfaces.

The various subtypes of leukemias laboratory researchers have uncovered using flow cytometry do not merely satisfy the scientific instinct to order and classify, but rather are revolutionizing the care of leukemia patients. Studies reveal that effective treatment of some of these subtypes require higher doses or more extensive or prolonged chemotherapy regimens. The leukemia cells of T-cell ALL, for example, tend to suck up methotrexate less avidly than those of B-cell ALL, so higher doses of this drug are needed to effectively treat patients with T-cell ALL. Other patients have leukemia subtypes that are so resistant to any chemotherapy that doctors take the more drastic measure of performing bone marrow transplants on them, as this risky procedure is their only hope for a cure. In contrast, some subtypes of B-cell leukemia easily succumb to chemotherapy in children, who therefore require less than the two or three years of chemotherapy that is standard for other types of leukemia.

Detecting lurking leukemia

When to stop standard chemotherapy is actually one of the more thorny decisions doctors have to make when treating leukemia patients. If they administer a group of highly toxic drugs longer than is necessary, they could subject their patients to life-threatening side effects. But if they stop too soon, the leukemia could return with a vengeance. What these doctors need is a reliable way to detect any lingering leukemia cells in their patients' bone marrow.

For example, traditionally patients with ALL were considered to be relatively free of leukemia if microscopic examination of their bone marrow revealed a normal number of immature B or T cells—no more than five cells per hundred. Doctors would often stop chemotherapy in patients once they reached this goal. But the leukemia would quickly come back in many patients who satisfied such crude criteria as each of these patients could still harbor as many as a billion leukemia cells in their bodies.

In contrast to this insensitive microscopic means for detecting leukemia cells in the bone marrow, flow cytometry can detect the elusive "needle in the haystack." Because doctors can often use this procedure to detect the distinctive cell surface signatures of their patients' leukemia cells, they can pinpoint a single leukemia cell in a sea of ten

thousand normal white blood cells. If such leukemia cells are detected after a bout of treatment, then the patient usually receives another round of more aggressive chemotherapy.

Greater sensitivity in the detection of leukemia cells has also come from a technique known as polymerase chain reaction (PCR). This revolutionary technique was first conceived of in 1985 by the biochemist Kary Mullis of a small California biotechnology company. (See the *Breakthroughs in Bioscience* article on PCR called "The Polymerase Chain Reaction," which can be found online at http://www.faseb.org/opar/break/).

The PCR technique that Mullis created does its molecular detective work by using genetic probes that seek out a specific genetic sequence. Bacterial enzymes are then used to copy the desired sequence repetitively until it is abundant enough to be detected by various laboratory equipment.

PCR has been used to reveal the specific genetic flaws harbored by leukemia cells and can detect a single leukemia cell among nearly a million normal bone marrow cells. Like flow cytometry, PCR can be used to fine-tune diagnosis and detect leukemia cells that remain after treatment. This allows doctors to custom tailor leukemia therapy so it is a better fit for the types of leukemia their patients have.

Indeed, the ability to use PCR, translocation analyses, or flow cytometry to home in on the precise defects that trigger various leukemias is a fitting end to a medical detective story that began with the crude observations of Virchow about 150 years ago.

Thanks to a succession of curious scientists from a diverse array of fields, including laboratory researchers as well as clinicians, leukemia has gone from being a mysterious disease, to being a group of well-defined disorders. By zooming in from the gross abnormalities of these leukemias to leukemia cells' molecular fatal flaws, these researchers, from the bench to the bedside, are gathering information that is blossoming into cures for many afflicted with these different types of bone marrow cancers. Because of their combined efforts, leukemia wards have gone from being the last way stations for a fast-approaching death train, to being depots of hope—places where doctors can often ensure that children and other leukemia patients have the long lives they deserve.

Biographies

Margie Patlak writes about biomedical research and health from the Philadelphia region. She has written for Discover, Glamour, Physician's Weekly, Consumer Reports on Health, The Washington Post, the Los Angeles Times, the Dallas Morning News, and numerous other publications. She also writes frequently for the National Institutes of Health and the National Academy of Sciences. This is her second article for the Breakthroughs in Bioscience series.

Alan S. Rabson, M.D., is the acting director of the National Cancer Institute at the National Institutes of Health. He came to the NIH in 1955, as a resident in pathologic anatomy. In 1975, he was named director of NCI's Division of Cancer Biology, Diagnosis, and Centers, where he served until his appointment as the institute's deputy director in 1995. Rabson holds clinical professorships in pathology at Georgetown University Medical Center and The George Washington University in Washington, D.C., and at the Uniformed Services University of the Health Sciences in Bethesda, Md. In 1987, Rabson became a member of the Institute of Medicine.

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