

**CHAPTER TWENTY-THREE**  
**THE FUTURE**  
**Looking For The Magic Bullet**

By Drs. Aubrey Pilgrim, Haakon Ragde, Gerald Murphy and E. David Crawford

When the sperm and egg unite, they form a single complete cell. This one cell has about 100,000 genes in its 46 chromosomes that will determine all of the characteristics of the new organism such as the color of the eyes, hair, body shape and many of the other inheritable traits. Of course, half of the genes came from the father's sperm and half from the mother's ovum. There will also be genes that were inherited from your grandparents and from their parents all the way back to that first little cell billions of years ago. Of course some of the genes from that first little cell have mutated and changed, but basically, you still have some components from that first little cell. The one main component that you inherited was life. The life in your body is the same life that originated with that first little cell about 4 billion years ago.

After the sperm and egg combine to make a complete cell, from this one cell, trillions of cells will develop. They will become cells that form arms, legs, eyes, skin or any of the other tissues that make up our body. During the very early embryonic stage of development, any cell can become whatever type of tissue that is needed. After all of the major structures have begun to form, the fetal cells lose the ability to become any other type of cell or tissue.

When a lizard and some of the other lower animals lose a part of their tail or even a foot, they can grow it back. It seems that they retain the ability for certain cells to become whatever is needed, very similar to those early embryonic cells. It is hoped that with enough research, we might be able to recover this embryonic capacity. It is believed that fetal tissue research can lead to many new treatments. Perhaps someday we can even grow a new prostate to replace a cancerous one that has been removed.

For several years, fetal research was banned. The ban has been lifted to some extent, but there are still several restrictions that hampers research. There are many people who are afraid that unlimited fetal research would lead to deliberate pregnancies then having it aborted for research. Of course this could not happen. For one thing, laboratories are forbidden from paying for any human tissues.

**Genes**

A tremendous amount of genetic research is being done. The Human Genome Project is very close to mapping all of the millions of genes in a human body. Even now with what has been learned we can detect certain abnormal genes inside the cells. The abnormal genes are known to be associated with the causation of certain cancers. Some of these abnormalities develop during the life of a cancer patient due to environmental toxins, injuries and just plain old age.

Tumor Suppressor Genes control and regulate the growth and multiplication of cells. They can detect a cell that has been damaged and kill it off. They can prevent cancers from

happening. If these genes don't function properly, cells may grow without a control and cause cancer.

Some of the tumor suppressor genes are the RB gene. If this gene becomes abnormal, it can lead to the development of Retinoblastoma, Bone, Breast, Lung, Prostate, Bladder and other cancers. The p53 suppressor gene can arrest replication of cells with damaged genes until normal repair process has taken place. If this gene becomes abnormal it can lead to the development of Breast, Colon, Leukemia, soft tissue sarcomas, and many other cancers.

If BRCA1, located in chromosome 17, and BRCA2; located in chromosome 13, becomes abnormal, there can be a very high risk of developing Breast cancer.

Over 100 oncogenes have been identified. They are essential components of normal genes within the cells. They don't cause any problems unless they become activated. We don't know why they become activated, but we do know that the many carcinogens that constantly bombard us may be a large factor. When activated, they can cause cancer by stimulating overactive cell growth.

From the time that the sperm and ovum unite, there is fantastic growth activity. When the person reaches adulthood, the cell growth is sharply reduced to replacing worn out or damaged tissue. There are certain growth hormones that regulates cell reproduction. Eventually we may be able to use these hormones to control cancer reproduction.

### **Gene Therapy**

Genes play a significant role in development of cancers. Genes carry the vital information about the cells and pass it on when a new cell is created. But a mutation due to any number of causes can ultimately lead to the production of different cells. There are many types and forms of genetic disorders. Gene replacement therapy has been successful in a handful of conditions, whereby an abnormal gene is substituted with a normal one. The process of substituting abnormal genes is a very difficult and complicated one, but they are learning more and more every day.

### **The Immune System**

The immune system is made up of several tissues, organs and physiological processes used by the body to identify proteins as abnormal or foreign. It then helps to prevent it from harming the body. When the body is exposed to or invaded by any of several different diseases and substances, it causes a reaction. The reaction causes antibodies to be created that are specific to that substance. If exposed to that particular substance in the future the antibodies will often be able to protect the body by destroying the substance.

The immune system is primarily composed of a group of cells, the lymphocytes, monocytes, and macrophages. The thymus is a part of the lymph system and it produces T cells. T cells function in the initiation and amplification of antigen-specific immune response. The T cells also function as cytotoxic killers of abnormal cells. The bone

marrow is also a part of the lymph system and it produces B cells. The B cells express immunoglobulin, a substance that is capable of acting as an antibody. Plasma cells are derived from the B cells. Macrophages, (macro- is from Greek macros for large, -phage is from Greek phagein, meaning to eat), are large cells that seek out any abnormal cells and literally eat them. The macrophages also process antigens and present them to T cells which helps activate the immune system.

Our immune system should be able to detect any defective cells and eliminate them immediately. We know that it does that to many cells. But some cancer cells seem to be able to escape the detection of the immune system. For some reason the body accepts them as perfectly normal cells.

The National Cancer Institute (NCI) and several other researchers are working on systems that would use modified genes to help fight cancer. NCI has found a gene that produces a protein substance that stimulates the body to fight off melanomas, the type of cancer caused by moles. But again and again, the first rule in dealing with cancer is that there are no rules. All cancers are different, so this same gene is not necessarily effective against other types of cancer. But this finding raises the hopes that there might be others.

You can read about some of the gene research at:

<http://www.ncbi.nlm.nih.gov/SCIENCE96/> and

<http://www.ncbi.nlm.nih.gov/genemap99/>

The Human Genome Project is expected to produce a sequence of DNA representing the functional blueprint and evolutionary history of the human species. However, only about 3% of this sequence is thought to specify the portions of our 50,000 to 100,000 genes that encode proteins. Thus an important part of basic and applied genomics is to identify and localize these genes in a process known as transcript mapping. When genes are expressed, their sequences are first converted into messenger RNA transcripts, which can be isolated in the form of complementary DNAs (cDNAs).

A small portion of each cDNA sequence is all that is needed to develop unique gene markers, known as sequence tagged sites or STSs. These sites can be detected in chromosomal DNA by assays based on the polymerase chain reaction (PCR).

You can find out more at: <http://www.ncbi.nlm.nih.gov/> or

<http://www.ncbi.nlm.nih.gov/cgi-bin/SCIENCE96/genelist>

You should be aware that most of the material at these sites are for scientists who are studying cancer at the molecular and cellular level so it may be a bit over the average persons head.

### **Coffee Break**

Here is some information about Coffee Break from

<http://www.ncbi.nlm.nih.gov/Coffeekbreak/Archive/about.html>

Coffee Break is a collection of short reports on recent biological discoveries. Each report incorporates interactive tutorials that show how bioinformatics tools are used as a part of the research process

Following the success of both molecular biology and information technology has risen a new and rapidly evolving discipline: bioinformatics, the process by which data is gathered, organized and computationally analyzed. Bioinformatics is really a theoretical approach to biology that makes use of computer algorithms and existing knowledge to mine nuggets of useful information from the mass of molecular data available. This approach can provide scientific investigators with new leads to solve biomedical problems - there are few, if any, experiments undertaken these days that do not involve some element of online data analysis, ranging from literature and DNA sequence searches to 3D molecular modeling.

The National Center for Biotechnology Information (NCBI) is a part of the National Library of Medicine, at the National Institutes of Health. Its mission is to organize DNA sequence and related data in such a way that it is available for investigators to use and analyze. This has involved the provision of tools such as BLAST, the sequence similarity search tool, and the design of search engines, such as PubMed, for mining the biomedical abstracts database, MEDLINE.

### **Future of Genetics**

The Human Genome Project is entering its climactic phase and should be completed as early as 2003 or earlier. It will be a complete and accurate DNA sequence representing the genetic blueprint and evolutionary history of the human species. Moreover, a "working draft" of this "book of life" may be available as early as 2001 and, in both cases, an index to the chapters and paragraphs will greatly enhance both the completion of the finished product as well as the practical utility of the intermediate results for biomedicine.

This new gene map represents such an index as it includes the locations, within this text, of more than 30,000 genes and provides an early glimpse of some of the most important pieces of the genome. Even more importantly, the map can be immediately applied by scientists to the identification and isolation of genes that either directly cause human ailments or increase our susceptibility to disease.

When they complete the Human Genome Project we will have an overwhelming wealth of information. Already some doctors and scientists are using what knowledge that we have at the moment to do genetic testing and counseling. By looking at the genetic makeup of a person, they can predict with a fair amount of certainty what diseases that person may contract. Right now they can predict whether a woman may eventually have breast cancer and some of the other diseases.

Is knowing the risk of eventually having cancer a good thing? In one way yes, the person can take steps to prevent it from happening. But it may cause untold anxiety. It may also lead to discrimination. If an insurance company knows that a person is predisposed to

having cancer, they would not want to insure the person. Also employers might not want to hire a person who was at risk of having a major disease.

This government research will help us all. Our only complaint should be that there is not enough research. We need to spend a lot more money to win the war on cancer.

## **Clinical Trials**

In order for a drug to be approved by the FDA, it must go through several slow and expensive steps. At one time it was fairly easy to obtain FDA approval. In the 1950s and 1960s, a drug called thalidomide was found to have many good properties as a pain reliever and sedative. Several pregnant women took the drug with disastrous results. Babies were born without limbs and other horrible and severe birth defects. It was later found that the thalidomide prevented the growth of blood vessels which prevented the development and growth of limbs in the embryos.

Cancer needs lots of blood, so it expresses substances that cause the body to build new blood vessels, called angiogenesis. Thalidomide and some other drugs can inhibit angiogenesis.

Since the thalidomide tragedy the FDA has become very cautious. Some people believe that they have become overly cautious. For instance there are drugs used in other countries that are making life more endurable and even saving lives of cancer victims. The so-called abortion pill, RU-486 would seem to have some very good cancer fighting properties. It has been used in Europe for some time. It has finally been allowed in this country, but it must go through the time consuming and costly expense of being tested just as if it were a brand new unknown drug.

It seems rather paradoxical and incongruous that the Government worries about the chances of a dying person having some minor side effects of a drug.

## **Angiogenesis**

When a tumor is first formed, it will live off the existing blood supply to the normal cells. The blood brings nutrition to all cells, cancerous and normal. The blood and lymph systems then pick up the waste products and remove it from the area. But if the tumor grows to be more than just a few millimeters in size, it will not be able to get enough nutrition from the existing supply to continue growing. So the tumor either remains as a small harmless entity, or it causes the body to lay down new blood vessels just so it can continue to grow. Our obliging body builds the blood vessels, yet doing so can lead to the death of the host.

Most tissues and cells in our body wear out and are replaced. This includes the individual cells that make up blood vessels. Usually an individual cell may live two or three years before dying off and being replaced. But blood vessel growth becomes very rapid in healing wounds and injuries, in embryonic development and for tumors.

Factors have been detected in cancer cells that cause blood vessels to be formed for their benefit. The substance has been called angiogenetic factors. (Angio is a prefix for blood or lymph vessels and genesis means birth or generation). Not only do the blood vessels provide needed nutrition, they also offer an exit path for the cells to migrate and set up new colonies.

Some doctors have claimed that they can make a fairly accurate prediction as to whether a prostate cancer has metastasized by counting the number of blood vessels in a sample of the tumor.

A group of scientists at the Judah Folkman laboratory have discovered a couple of substances they call angiostatin and endostatin. These factors act the opposite of the angiogenetic factors by preventing the establishment of new blood vessels. If a cancer can be prevented from growing more than a tenth of an inch in diameter, or about the size of a drop of water, it would probably never cause any problems.

The Angiostatin protein is already made in the human body to a small degree. It attempts to block the growth of diseased tissue, but isn't always successful, especially in severe cases. When the body is given additional Angiostatin protein, however, this naturally occurring antiangiogenic agent can block the growth of tumors by depriving them of their blood supply.

This protein was discovered in 1994 by Dr. Michael O'Reilly and Dr. Folkman's team of researchers at Children's Hospital, in cooperation with Entremed. In pre-clinical studies, tumors of the breast, prostate and colon significantly regressed when deprived of their blood supply through treatment with this natural antiangiogenic protein. Endostatin is another antiangiogenesis protein that was discovered in Dr. Judah Folkman's laboratory.

The Entremed Company has a web site at [www.entremed.com](http://www.entremed.com).

There are about 8 million survivors of all types of cancer. In some cases, the cancer has gone into a spontaneous remission. No one knows why some cancers go into remission. It could be that some people have a better immune system than others. It could also be that their bodies produced enough angiostatin to starve the cancers to death.

There are several other companies who are developing antiangiogenetic substances. SUGEN at [www.sugen.com](http://www.sugen.com) is just one of them.

When tumors grow to a certain size (2-3 mm) and become hypoxic, or oxygen starved, they synthesize and secrete vascular endothelial growth factor (VEGF). This growth factor is a type of molecular messenger, sending a signal to nearby blood vessels that triggers the activation, division, and migration of endothelial cells that line the blood vessel walls. This process results in the sprouting of capillaries from the blood vessels and into the VEGF-secreting tumor.

The signal transduction process that results in the endothelial cell activation is mediated by receptors on the endothelial cell surface. SUGEN's proprietary transcript imaging technology for gene expression analysis has shown that endothelial cells express a large number of tyrosine kinase receptors (TKs). SUGEN's target validation studies have implicated one of these TK receptors, Flk-1, the receptor for VEGF, a key regulator of the signaling cascade that leads to endothelial cell division and angiogenesis. These target validation studies have implicated Flk-1 as a primary driver of angiogenesis in the majority of solid tumors.

The pharmaceutical industry has long sought small molecule inhibitors of angiogenesis with low toxicity profiles. The inhibition of angiogenesis may limit tumor growth, extend the period of disease-free remission in patients who respond to front-line therapy, and reduce the potential for metastases.

## **Vaccines**

### **by Dr. Haakon Ragde And Gerald Murphy**

Dr. Gerald P. Murphy was the principal investigator and scientist in the development of the dendritic vaccine. Dr. Murphy was Director of Research, Pacific Northwest Cancer Foundation, Cancer Research Division, Northwest Hospital, Seattle, WA; and Editor in Chief of CA Journal. Dr. Murphy was one of the pioneers who first studied PSA. He made an enormous contribution to prostate cancer research and treatments.

Dr. Murphy died on January 21, 2000.

The human body is protected from outside invaders by a complex network of specialized cells (which include T cells and B cells), and are known as the immune system. This network can destroy various pathogens (for example, different strains of bacteria) which enter the body. Cells involved in the immune function of the body recognize components of these foreign intruders (known as antigens) and neutralize them. The system also has a distinctive property called "memory", allowing for a more effective response to pathogens after previous exposure. This property serves as a basis for vaccination procedures which are a potent guard against various pathogens.

The utilization of immune system enhancement as an adjunct of cancer therapy is a rapidly growing field. Development of a vaccine for cancer is an idea pursued for decades only to meet with limited success to date. However, during this period of time we have gained a great deal of knowledge in several areas which are crucial to the successful development of a cancer vaccine.

Firstly, in the field of tumor biology, specific proteins expressed by tumor cells (known as tumor antigens) are being identified and characterized for further use as targets for immune attack. An example of such an antigen expressed by prostate cells is the prostate- membrane antigen (PSMA). Secondly, several other significant findings have been made in the field of immunology. For example, specialized cells were identified as special tools of the immune system known as antigen presenting cells (APC). These cells possess the capability to bring antigens inside them and cut them into small fragments

known as peptides. These peptides are bound to human leukocyte antigen (HLA) proteins, and returned to the cell surface.

Recognition of these peptide/HLA complexes results in activation of one of the important components of immune system known as T cells. Dendritic cells have been identified as the primary antigen presenting cells. In the present report we will briefly review the use of dendritic cells and the prostate specific membrane antigen (PSMA) in the development of a prostate cancer vaccine.

### **Prostate Specific Membrane Antigen**

Prostate specific membrane antigen was discovered in 1987 by Horoszewicz, Kawinski and Murphy. This protein is localized on the cell membrane, specific to prostate and is over expressed in prostate cancer. Israeli and colleagues cloned the PSMA gene in 1993. This protein consists of 750 amino acids.

### **Dendritic Cells as Antigen Presenting Cells**

Dendritic cells are the most potent antigen presenting cells known because they express various components required for an effective T cell activation. Their origin and lineage are not known. Small numbers of dendritic cells can be isolated from various tissues including spleen, bone marrow, lymph nodes, skin and peripheral blood. The key to dendritic cell use and study was the ability to increase their number by culturing them for an extended period of time. Our solution was to use peripheral blood as a source of dendritic cells and the use of factors known as granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin 4 (IL4). This combination of factors allows for expansion and survival of dendritic cells for at least two-three weeks in culture. At least ten million dendritic cells can be cultured from 100 ml of blood. Cultured dendritic cells can process antigens and successfully activate T cells.

### **Development of a Prostate Cancer Vaccine**

Two major components comprise our prostate cancer vaccine. The first component is dendritic cells, which are isolated from the patient and cultured for purposes of expansion. We have successfully shown that specific T cells can be activated in a tissue culture dish, when patients' white blood cells are cultured with the same patients' dendritic cells which have been incubated with tumor cell lysate. These activated T cells can furthermore kill patients' tumor cells in a tissue culture dish.

In order to gain simplicity in the prostate cancer vaccine and avoid the generation of unwanted nonspecific T cells, we have carried out experiments using two PSMA peptides (9 amino acid long), which preferentially bind HLA-A2, an HLA-type which is expressed by a large portion of the population. These peptides, after incubation with patients' dendritic cells, are able to activate the same patients' T cells in a test tube. Additional peptides from PSMA and other antigens are currently being tested in order to establish a cocktail of peptides for the second component of our prostate cancer vaccine. Thus, the concept of our vaccination protocol involves infusion of patients' dendritic cell/peptide combination back into patients.

The advantages of a dendritic cell based prostate cancer vaccine are several fold. First, no foreign genes or viral vectors are introduced into cells as in gene therapies. Second dendritic cells express as the necessary components for an effective activation of T cells, eliminating the need to expose patients further to additional cytokines. Third, all procedures are carried out with the patients' own materials, including dendritic cells and plasma, thus eliminating unwanted non-specific responses.

A phase one clinical trial assessing the administration of patients' own dendritic cells pulsed with PSMA peptides was carried out by Northwest Hospital. Fifty one patients with advanced metastatic prostate cancer participated in this study. Two distinct HLA-A2-specific PSMA peptides were selected for this study (PSMA peptides 1 and 2). Participants were divided into five groups receiving 4 or 5 infusions of test substances. No significant toxicity was observed in any of the five groups. T cell response was observed in HLA A2-positive patients infused with dendritic cells pulsed with either PSMA peptides 1 or 2. An average decrease in PSA (prostate-specific antigen), a measure of positive response, was detected in HLA-A2-positive patients infused with autologous dendritic cells pulsed with PSMA peptides 1 as well as 2. Seven partial responders were identified based on National Prostate Cancer Project (NPCP) criteria+ > 50% PSA.

Dendritic cells (DC) are considered the most potent APC of the immune system, and are unique in their ability to stimulate naïve T cells. DC are adapted to capture proteins, proteolytically digest them, and present the resulting peptides on their cell membranes bound to MHC antigens. Formation of this MHC-peptide complex is crucial to the activation of T cells. In addition, DC express high levels of the costimulatory molecules, CD80 and CD86, which are required for full T cell activation. DC are found in the epidermal layer of the skin, the respiratory and gastrointestinal systems, and the interstitial regions of several solid organs where they function as sentinels, capturing invading microorganisms for presentation to immune cells. Until recently, the study of DC was limited because few cells could be isolated from tissues or peripheral blood. With improvements in DC isolation and culture techniques, much larger numbers of DC are available and immunotherapy using DC is now feasible. DC can be derived from peripheral blood using cytokines, such as GM-CSF, IL-4, and tumor necrosis factor-alpha (TNF-alpha).

DC-based cancer vaccines have recently been tested with some success in clinical trials with several types of cancers, such as follicular-B cell lymphoma and melanoma. Our group has been developing a prostate cancer vaccine using autologous DC as a vehicle to present prostate antigens to T cells in vivo. Our phase I and II clinical trials in prostate cancer with DC pulsed with HLA-A0201-specific prostate-specific membrane antigen (PSMA) peptides will be discussed in this report.

### **Prostate Cancer Vaccine**

Two major components comprise our new prostate cancer vaccine. The first component is DC, which are isolated and cultured from patient peripheral blood. The second component is a specific antigen used to target prostate cancer tissues. Few antigens

specific to prostate tissue have been characterized for use as target antigens for immunotherapy. This short list includes prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA).

PSMA is a 750 amino-acid membrane-bound protein expressed by prostate epithelial cells. It has been utilized diagnostically as part of a prostate cancer imaging method (ProstaScint<sup>®</sup>, Cytogen Corp., Princeton, NJ), which uses a monoclonal antibody specific for PSMA (7E11.C5.3). Enhanced expression of PSMA was detected in hormone refractory prostatic carcinoma. In addition, levels of PSMA are elevated in the sera of hormone refractory advanced prostate cancer patients. Our immunotherapy protocols use PSMA as target antigen for T cell attack in vivo.

At the time of this writing, late 2000, Dendritic Vaccines are now in Phase III trials. The vaccine has been taken over by the Dendreon Corporation.

The double blind, placebo-controlled trials will seek to confirm the results obtained in earlier studies, which suggest the effectiveness of Dendreon's immunotherapy in delaying the progression of prostate cancer.

Dendreon Corporation is at <http://www.dendreon.com/>

For more information about the clinical trials, please call (206) 256-4545. Fax: 206-256-0571 3005 First Ave Seattle WA 98121

### **Other Research on Vaccines**

A team of Johns Hopkins Oncology Center researchers has developed a vaccine that helps strengthen the body's immune system against prostate cancer, according to a study published in the journal Cancer Research.

Several other studies are also being conducted by many companies and institutions.

### **A Bit Of Trivia**

The word vaccine is from the Latin vaccinus, pertaining to cows. Some of the early research involved using cows.

Mr. C. Davenport posted on the Internet a bit more about the origins of vaccines: "The term, vaccine, indeed comes from Latin and refers to cows. The first safe and effective vaccine consisted of material from crusts of the hands of Sarah Nelms, a milkmaid suffering from cowpox, a relatively mild disease transmitted to humans from cows.

In 1796, Dr. Edward Jenner, an English country physician, transferred material from Sarah's cowpox lesions to James Phipps, a healthy 8 year old who had never had smallpox. Shortly thereafter, he twice challenged James with variolation (inoculation with material from smallpox lesions) and found that James was completely immune.

Variolation had been practiced for many centuries in Africa and Asia, and had been introduced to Europe in the late 1700's. (Catherine the Great of Russia and Louis the XVI

of France underwent variolation). Variolation, however, was not always safe and resulted in active smallpox in approximately 1 to 2 % of those treated.”

### **Exisulind or Aptosyn**

Here is some information from Cell Pathways Inc. at <http://www.cellpathways.com>  
They are pioneering a new approach to the treatment and prevention of cancer with the development of agents termed Selective Apoptotic Antineoplastic Drugs (SAANDs). Living cells typically die by one of two mechanisms (necrosis or apoptosis). Necrosis refers to traumatic cell death resulting from, for example, a severe burn or injury. Necrosis is usually caused by an external force. Apoptosis, or programmed cell death, refers to an orderly sequence of responses to biochemical or physical signals that end in a cell committing suicide. This is the body’s attempt to get rid of unwanted or damaged cells. It is known that all cells have the capacity to die by this process.

Selective induction of apoptosis in precancerous and cancerous cells is an approach to the treatment and prevention of cancer in which only precancerous and cancerous tissue is directed to self-destruct, with no impact on normal healthy tissue.

Cell death is normally a tightly regulated process in which cells are constantly reacting to chemical signals from other cells or from their environment instructing them to either live or die. If the balance between signals instructing cells to live or die is lost, disease or death may result. For example, in degenerative diseases such as Alzheimer’s disease, too many brain cells die inappropriately. In cancer, cells lose the ability to recognize the signal to die, which results in cellular "immortality" and uncontrolled growth and spread. Cancer therapies are designed to arrest and kill cancer cells. However, traditional chemotherapy and radiation therapies nonselectively kill all rapidly dividing cells, which include both healthy and cancerous cells. Thus, the usefulness of radiation and chemotherapy is limited because of their toxicity to healthy cells.

SAANDs take advantage of a defect that is present only in precancerous and cancerous cells, which accounts for the selective nature of the drugs for only abnormal tissue. This defect appears to occur early in the multistage process of the development of progression to cancer and is also apparent in cancerous tissue. Therefore, research has suggested that Cell Pathways’ SAAND technology acts at both the precancerous and cancerous levels of the continuum.

In cancer, the genetic and chemical messengers that mediate the balance between the rates of cellular proliferation and apoptosis are damaged, resulting in cellular "immortality" and uncontrolled growth. Cell Pathways’ research has led to an understanding of one of these imbalances and has identified a new pathway for triggering apoptosis in precancerous and cancerous cells. This new pathway is based upon the increased activity of a protein, a novel cyclic GMP phosphodiesterase (cGMP-PDE). Cyclic GMP (cGMP) is important in the cellular mechanism for apoptosis. By inhibiting the enzymatic breakdown of cGMP (via cGMP-PDE), apoptosis signals may proceed normally in cancerous cells.

In normal cells, the presence and activity of this novel cGMP-PDE are minimal, and the signals that trigger apoptosis initiate a sequential series of steps in the pathway, culminating in apoptosis. At one point in the pathway, apoptosis signals are conveyed via cGMP, a key intracellular signaling molecule. The apoptosis-triggering signals cause a rise in cellular cGMP that activates the next step in the pathway.

By contrast, in precancerous and cancerous cells, there is a substantial increase in cellular cGMP-PDE activity. Cyclic GMP-PDE degrades cGMP within the cell. The increased activity of cGMP-PDE prevents the normal rise in cGMP and interferes with the apoptosis pathway. SAANDs act by specifically inhibiting the activity of the newly discovered cGMP-PDE, thereby permitting a subsequent rise in cGMP levels. Thus, SAANDs allow the apoptosis pathway to again function normally.

Recent studies by Cell Pathways in conjunction with various universities demonstrate that Aptosyn™, as well as other SAANDs synthesized by Cell Pathways, induces apoptosis in a variety of cancer cell lines. One study in both androgen-sensitive and androgen-insensitive prostate cancer cell lines showed that Aptosyn induced apoptosis in a time- and dose-dependent manner. Another study found that Aptosyn induced apoptosis in cell cultures of human leukemia and myeloma. Additionally, a collaborative project with a different university found that Aptosyn demonstrated synergistic activity when administered in combination with cis-retinoic acid or nordihydroguaiaretic acid (two potential chemopreventive agents), as well as with chemotherapeutic agents such as cisplatin or paclitaxel. Using Aptosyn in combination with cisplatin and paclitaxel, strong synergistic activity was observed at concentrations that were subtherapeutic for each drug alone.

A study of Exisulind (brand name Aptosyn) from Cell Pathways Inc (CPI) involved 92 evaluable patients in a randomized double blind, placebo-controlled multi-center trial. Forty seven men received placebo and 45 received Aptosyn. The rise in average PSA levels in the Aptosyn treated group was significantly lower than that of the placebo-treated group. If patient groups were stratified into high, intermediate and low risk groups for developing metastases, Aptosyn helped the most in the high-risk group with the intermediate group nearing statistical significance. Note, Aptosyn is not a COX I or II inhibitor but a cyclic GMP phosphodiesterase inhibitor that selectively induces apoptosis in abnormally growing precancerous and cancerous cells but not in normal cells. Cell Pathways is working on a new SAAND drug called CP-461; it is in Phase I trials now. There may be more information on these agents at the website:  
<http://www.cellpathways.com>

Note that Viagra is also a phosphodiesterase inhibitor. It is a PDE type 5. There are several types of PDE from type 2 up to type 11. Viagra may be effective for two to four hours. Aptosyn may be effective up to 24 hours. Viagra and Aptosyn prevents or inhibits the PDE from degrading the cGMP.

## **High Intensity Focused Ultrasound-**

High Intensity Focused Ultrasound (HIFU) called, Ablatherm, appears to be a new type of therapy that is very minimally invasive. This technology utilizes a focused ultrasound beam to destroy well-defined tumors without affecting the surrounding tissue. The device is currently being used in a five year-old multicentric study in Europe to treat localized prostate cancer. Over 300 patients have already been treated with promising results.

HIFU represents a valid alternative treatment strategy for patients with localized prostate cancer, who are unsuitable for surgery. With the Ablatherm treatment there is no incision, no needles and no radioactive seeds.

For more information in the U.S., contact EDAP Technomed, the Company's U.S. subsidiary located in Atlanta, GA, by phone at (770) 446-9950 or on the World Wide Web at [www.edaptechnomed.com](http://www.edaptechnomed.com).

We still have not found the Magic Bullet for prostate cancer. But we will eventually. We could find it a lot sooner if we had more money for research.

**The answer to cancer is early detection**