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Review Article

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The Impact of Sequencing Human Genome on Breast Cancer

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ABSTRACT

An estimated 42,140 women in the U.S. die from breast cancer this year. Approximately 321,910 new cases of invasive breast cancer are expected to be diagnosed next year. Worldwide 670,000 to 764,000 deaths occur annually making it the leading cause of cancer death among women. Current treatments include surgery and chemotherapy. After surgery, the most widely used compounds for treating breast cancer are Taxanes and Anthracyclines. After the surgery and chemical treatment, the remaining malignant cells return in three years as metastatic breast cancer and kill the patients. Presently, there is no cure. If there is a family history of an inherited disease in a woman's family, several reproductive technologies can help prevent the transmission of genetic defects If your mother or her sister or any member of your family died of breast cancer, you are most likely to inherit the mutated BRCA1 or BRCA2 genes responsible for causing breast cancer. Now, we have sequenced the entire human genome, it is easy to identify the deleterious genes in embryo before conception. The following two genes are associated with breast cancer: **BRCA1:** gene is located on Chromosome 17 (at position 17q21). **Chromosome-17** is a very long chromosome. It is made of 92 million base pairs (AT & GC) and carries 1,394 genes. On the other hand, the **BRCA2: gene** is located on Chromosome 13 (at position 13q12.3). Chromosome 13 is also a very large chromosome and it is made of 114 million base pairs (AT & GC) and carries 496 genes. The only way to have children free from breast cancer is to conduct the genetic analysis of your embryo. Soon after conception, the fertilized ovum becomes an 8-cell embryo within 3-days. Each cell contains the complete genome. The genome is made of 4-nucleotides (AT & GC), the bio bricks of life. They are not alive because they can be frozen in liquid Nitrogen at 70 degrees below zero for years. When needed, it could be thawed and implanted in the womb. Once implanted in the womb, these bio bricks organized themselves to become alive. Sequencing each cell and comparing it with the Reference Sequence (described below) will help us eliminate any deleterious mutation. Only cells free from all mutations including BRCA1 or BRCA2 will be implanted. As the cost of sequencing is coming down, from the 8-cell embryo, we can sequence each cell cheaper and faster. The child born after the genetic analysis is sure to be free from Breast Cancer causing genes. The only guaranteed cure from breast cancer can be achieved by genetic analysis of the mother, her egg, her ovum and her embryo. The major source of transmission of breast cancer of mutated BRCA1 and BRCA2 from mother to daughter can be eliminated. Despite all precautions to remove deleterious mutations from the cell, we must continue to develop new drugs to treat breast cancer. Despite all precautions to remove deleterious mutations from the cell, we must continue to develop new drugs to treat breast cancer. There are other factors which cause breast cancer such as exposure to radiation, chemical or environmental pollution and viral infection.

Keywords: Brca1 Gene, Brca2 Genes, Taxanes and Anthracyclines, Nitrogen Mustered, Aziridines, Carbamate, Azq.

Background Information

Breast cancer has been documented since antiquity, with the earliest known account appearing in the Egyptian Edwin Smith Papyrus around 1600 B.C.E. For thousands of years, treatments were strictly palliative and focused on humoral theories until scientific milestones transformed medical care. Modern view is that only 5–10% of all cancer cases can be attributed to genetic defects, whereas the remaining 90–95% have their roots in the environment and lifestyle.

Inherited gene mutations can increase the risk for specific types of cancers (such as BRCA1/2 genes for breast/ovarian cancer or Lynch syndrome for colorectal cancer.

Approximately 90% to 95% of all cancers are not hereditary. Instead, they are sporadic, meaning they are caused by acquired gene mutations from aging, lifestyle, or environmental factors like tobacco smoke or UV radiation. Common examples include lung cancer, cervical cancer, skin cancers (like melanoma), and liver cancer. The initiation of breast cancer is due to transforming (genetic and epigenetic) events in a single cell. Subsequent tumor progression is driven by the accumulation of additional genetic changes combined with clonal expansion and selection.

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Based on these assumptions several groups have performed comprehensive gene expression and genetic profiling studies comparing in situ, invasive, and metastatic breast carcinomas but have failed to identify tumor stage–specific gene signatures. However, these studies have focused mainly on tumor epithelial cells, while the potential involvement of other epithelial and myoepithelial cells and the stroma in tumor progression have not been explored in sufficient depth. Breast cancer is caused by genetic mutations that prompt breast cells to grow and divide uncontrollably, eventually forming a tumor. While the exact trigger for every case is unknown, it generally results from a complex mix of genetics, hormones, lifestyle habits, and environmental factors

Ross' Rationale for using War chemicals to treat Cancers

Soon after the discovery of double helical structure of DNA by Crick and Watson, Professor WCJ Ross (a true intellect) of London University realized that during replication, the double strand DNA separates and each strand take the complimentary nucleotide forming two cells [1]. Since the cancer cells divide faster than normal cells by cross-linking both strands of DNA. He could prevent the replication of cancer cells.

Nitrogen Mustard was mercilessly used as a weapon during the WWI by both German and Italian Armies against Allied forces. Most soldiers exposed to Nitrogen Mustard were freeze to death. Their blood analysis showed a sharp decline in White Blood Cell (WBC). Since patients with the cancer of the blood called Leukemia showed a sharp increase in WBC, Professor Ross and his group at London University, England, wondered if minimum amount of Nitrogen Mustard could be used to control Leukemia in cancer patients. It was indeed found to be true. During the following 30 years, Ross developed hundreds of derivatives of Nitrogen Mustard to treat a variety of cancers. His most successful drugs are Chlorambucil, Melphalan and Myrophine As his graduate student, during the following ten-year period, I made for Professor Ross dozens of analogs of Nitrogen Mustards. The deadliest among them was the Phenylenediamine Mustard. We use these compounds to check the sensitivity of the Experimental Tumors in the Tumor Bank. If tumors in the Tumor Bank become resistant, we must replace resistant tumor cells with fresh more sensitive tumors for testing other compounds.

Ross was the first person who conducted Radiolabeled study to show that Nitrogen Mustard shut off a gene by cross-linking both strands of DNA that we inherit one strand from each parent. It was the same Cross-linking agents such as Nitrogen mustard made by Haber. Solders exposed to Nitrogen Mustard showed a sharp decline of White Blood Cells (WBC) from 5000 cell/CC to 500/CC. Children suffering from Childhood Leukemia have a very high WBC count (over 90,000/CC). Most of the WBCs are premature, defected, and unable to defend the body from microbial infections. Ross rationale was that cancer cells divide faster than the normal cell; by using Nitrogen Mustard he could use cross linking DNA and prevent cell division. Once he demonstrated that he could shut off a gene by cross-linking DNA; he could shut off any mutated gene including the genes of all 220 tissues present in a human by finding a dye that could specifically color that tissue. He could attach the Nitrogen Mustard group to the dye and attack the cancer genes in any one those 220 tissues.

Reference Sequence

The Human Genome: The greatest Catalog of Human Genes on planet Earth

When we sequenced our entire genome, we read our book of life, letter by letter word by word, sentence by sentence, chapter by chapter all forty-six volumes (chromosomes) written in six billion four hundred million genetic letters (nucleotide) of a healthy human being under the Human Genome Project. We can use our healthy Genome as a Reference Sequence for comparison. Using Nano Capillary Sequencing method, it took us 13 years to sequence the entire human genome at a cost of $3 billion. Now, we have developed next generation sequencers like Nanopore technology which will sequence the entire genome cheaper and faster. Using biopsy sample, we can take a single cell from the Lung or Oral tumor of smoker, sequence its genome, and compare it with the Reference sequence to identify the number and location of all mutations or damage genes caused by smoking. Recently, we also completed the 1000-genome project which will provide thousand copies of the same gene sequence for comparison. We also learned to convert Analog language of Biology into the Digital language of computer. Now, we can write a program and design a computer to read and compare and send the data to any country in the world at the speed of light. When comparing with the Reference Sequence with the smoker's gene sequence, it will identify all the mutations with precision and accuracy. Once the mutations responsible for causing any cancer including Lung, or Oral Carcinoma are identified, we can design drugs to shut off those genes.

There are three billion two hundred million AT/GC base pairs in every cell in humans and are arranged like a spiral staircase. We inherit each strand of the staircase from each parent. All humans have 23 pairs of chromosomes. As I mentioned above, we look different because within every 1000 to 1300 genetic letters, one base pair is located at a different place called Single Nucleotide Polymorphism (SNP). In a three billion four hundred million base pair genome of a person, there are at least 3 million SNP which make us look different from one another.

Although all living creatures have the same AT/GC base pairs, the numbers of chromosomes are different in each species. A unit or a large number of AT/GC base pairs that are arranged to produce a protein is called genes (usually from a few hundred genetic letters to a few thousand.) When we eat food, each ingredient are broken down by proteins or enzymes produced by these genes to extract energy needed by our body. You can chemically connect AT/GC base pairs together.

We deciphered all 46 chromosomes, 23 from each parent. The 46 chromosomes present in each cell of our body are the greatest library of the Human Book of Life on planet Earth. The Human Genome Project has identified the following genes on each chromosome:

We found that the chromosome-1 is the largest chromosome carrying 263 million A, T, G and C nucleotide bases and it has only 2,610 genes. The chromosome-2 contains 255 million nucleotides bases and has only 1,748 genes. The chromosome-3 contains 214 million nucleotide bases and carries 1,381 genes. The chromosome-4 contains 203 million nucleotide bases and carries 1,024 genes. The chromosome-5

contains 194 million nucleotide bases and carries 1,190 genes. The chromosome-6 contains 183 million nucleotide bases and carries 1,394 genes. The chromosome-7 contains 171 million nucleotide bases and carries 1,378 genes. The chromosome-8 contains 155 million nucleotide bases and carries 927 genes. The chromosome-9 contains 145 million nucleotide bases and carries 1,076 genes. The chromosome-10 contains 144 million nucleotide bases and carries 983 genes. The chromosome-11 contains 144 million nucleotide bases and carries 1,692 genes. The chromosome-12 contains 143 million nucleotide bases and carries 1,268 genes. The chromosome-13 contains 114 million nucleotide bases and carries 496 genes. The chromosome-14 contains 109 million nucleotide bases and carries 1,173 genes. The chromosome-15 contains 106 million nucleotide bases and carries 906 genes. The chromosome- 16 contains 98 million nucleotide bases and carries 1,032 genes. The chromosome-17 contains 92 million nucleotide bases and carries 1,394 genes. The chromosome-18 contains 85 million nucleotide bases and carries 400 genes. The chromosome-19 contains 67 million nucleotide bases and carries 1,592 genes. The chromosome-20 contains 72 million nucleotide bases and carries 710 genes. The chromosome-21 contains 50 million nucleotide bases and carries 337 genes. The chromosome-22 contains 56 million nucleotide bases and carries 701 genes. Finally, the sex chromosome of all females called the chromosome-X contains 164 million nucleotide bases and carries 1,141 genes. The male sperm called chromosome-Y contains 59 million nucleotide bases and carries 255 genes.

If you add up all genes in the 23 pairs of chromosomes, they come up to 26,808 genes and yet we keep on mentioning 24,000 genes needed to keep us function normally. There are 16,000 good genes, 6,000 defected or mutated genes and 2,000 Pseudogenes. A gene codes for a protein, not all 24,000 genes code for proteins at the same time. (Alternative splicing could generate thousands of new combinations of proteins. (The cells use new combination to generate new proteins as needed). It is estimated that less than 19,000 genes are code for protein. Because of the alternative splicing, each gene codes for more than one protein. As needed by our body, a small number of genes interact to make specific proteins. All the genes in our body make less than 50,000 protein which interact in millions of different ways to give a single cell. Millions of cells interact to give a tissue and hundreds of tissues interact to give an organ and several organs interact to make a human [2-6].

The Human Genome is made of four nucleotides, the bio-bricks, of life. They are neither alive nor dead. They can be cut paste and copied. Out of four-letter text, three letters code for an amino acid called codon. Hundreds of codons join to code for a protein called gene. A gene is a unit of inheritance. Using Restriction Enzyme like EcoR1, we could cut the sequence at a specific site. We can move gene from man to mouse and from mouse to mosquitos and from mosquitos to microbes. Once in microbes, we can insert into a plasmid which acts as a vector which and carry the gene across the cell membrane into a bacterium where it cuts the bacterial genome at a specific site and insert the gene and enzyme DNA ligase join the plasmid with the bacterial genome. Bacteria replicate every 20 minutes. Within few days, it makes millions of copies of the gene.

The nucleus of every living cell carries complete genetic information to make a species. The basic flow of genetic information in any cell is as follows. The DNA replicates (makes its own copies). The information from DNA is transcribed or copied into a form known as "RNA". The complete set of RNA (also known as its transcriptome) is subject to some editing (cutting and pasting) to become messenger mRNA, which is translated in the ribosome, to its coded protein. DNA carries information, and the protein carries all the body function.

Total genetic information that makes a species is called its Genome. The book on life of a human being that is the Human Genome contains complete information to make us. As I said above, the entire Genome is written in four genetic letters AT/GC (these are the initials of four chemicals found in the nucleus of all living cells and they are Adenine, Thymine, Guanine and Cytosine) called the nucleotide. Sequencing Genome means reading the number and the order of the nucleotide in a genome and determining the genomic sequence, however, is only the beginning of genomics. Once this is done, the genomic sequence is used to study the function of the numerous genes (functional genomics), to compare the genes in one organism with those of another (comparative genomics), or to generate the 3-D structure of one or more proteins from each protein family, thus offering clues to their function (structural genomics). One of the most important reasons for sequencing a genome is to align the normal genome for comparison with the defected genome. The mutation in the defected genome is due to damage of DNA either by radiation, chemical pollutants, genetic inheritance, viral infection, insertion, deletion and inversion, rearrangements of DNA or SNP polymorphism. It identifies the markers for a particular disease. There are at least 6,000 diseases linked to genetic defects and more than 1,500 gene markers have been identified for different diseases.

Each cell in our body contains two genomes. One Genome within the nucleus and another is located outside the nucleus that is in the Cytoplasm. Both Genomes are present in every cell of our body except the Red Blood Cells (which contains no nucleus). The other Genome which is located outside the nucleus is the mitochondrial Genome. It is a prokaryotic Genome which is autonomous and replicated independently like bacteria. It is a bacterial genome and must have been captured over evolutionary time. It is the source of all energy for a living cell. While nuclear Genome is made of three billion two hundred million AT/GC base pairs and carries 24,000 genes, the mitochondrial Genome is made of 16,581 kilo-base AT/GC base pairs and carries only 13 genes. These genes carry energy containing ATP (Adenosine Tri Phosphate) molecules. The Phosphate bond is broken down to form Adenosine Di Phosphate releasing energy which is further broken down to Adenosine Mono Phosphate which is converted back to ATP in the presence of Phosphate and enzymes). Mutations mostly deletion of base pairs in mitochondrial genomes cause diseases. Less than two percent of the Nuclear Genome carries genes that are coding regions. More than fifty percent of the non-coding region contains transposable elements. Over evolutionary time, they must have been captured by other living creatures. They carry repetitive regions. They move around during replication. If any part of this element lands in the coding region, they act as switches and they will alter gene function.

There are Three Hidden Facts About our Genome

First, we have captured within the Genomes all the secrets of life of all species including information to make us. All good genes and bad genes are within our genome. Six thousand diseases due to mutations are within our genome. We have also captured secrets of life of all living creatures within their genomes. All the secrets are within the genomes, and these secrets can be experimentally tested. What is outside our genome? Outside our genomes are fictions which cannot be experimentally verified such as Magic, Mystic, Miracles, Life-after-Death, Holy Water, Hell and Heaven.

Second, we can compare the genetic profile of a healthy person with the sick person to see what variations are responsible for causing diseases. The Genome Wide Association Analysis (GWAS) shows variations in genome that can serve as a gene marker for identifying diseases. We should have our Genome sequenced when we are at the best of our health. Log our genome on our computer's default drive to use as a reference sequence for comparison. We should scan our Genome occasionally to see if there are any unusual changes for any upcoming diseases. We should start taking action to prevent diseases at their earliest.

Third, no two genomes are identical except the genomes of maternal twins. Because no two genomes are exactly the same, no two people look alike. If you compare the genomes of two individuals, we find one mutation in every 1,000 to1300 base pair (we call this variation, SNP, single nucleotide polymorphism). Since one SNP occurs roughly in every thousand base pair, in a three billion two hundred million base pair human being, there are at least 3 million mutations (SNP) that make us different from each other. If you are a religious person, you can say that since no two people look alike; we are all differently gifted. The individual purpose of life is to recognize the gift, master the gift and share the gift with the rest of the mankind, serve mankind and serve God that is the individual purpose of life of everyone on Earth. There is a higher purpose of life where we collectively save mankind from extinction.

The primary role of the genomes of all living creatures is to store and copy information so that living creatures reproduce the same species. Our appearance as humans, from a single living cell to a one hundred trillion cells creature has been the result of the four and a half billion years of biological evolution. By shuttling genes from one species to another, we have accelerated genetic evolution. Recombinant technology allows us to control the Darwinian Evolution. We are no longer passive observers of nature; we are active choreographers. Genetic engineering has allowed us to accelerate the evolutionary process. The variations that Nature has taken billions of years to introduce could be introduced in weeks.

Science tells us that every living thing on Earth, from a tiny blade of grass to the largest blue whale, is the result of a single experiment in life, the descendant of a single cell that developed four and a half billion years ago at some remote corner of the Earth. Life must have originated by the interactions of elements existing on Earth by natural processes obeying the laws of Physics and Chemistry and Darwinian Biological evolution, the basic elements on Earth organize themselves to become alive. Earth has a diameter of eight thousand mile, and its circumference is 25 thousand miles; every day a million lightning strikes on some parts of Earth. During the first billion years of its lifeless history, something important happened. Out of millions of lightning, a single lightning struck at a mass of gases containing a mixture of Carbon, Hydrogen, Oxygen, Nitrogen at a Phosphorus rock which reacted to generate the very first combination of atoms to form information molecules, a nucleotide. Polymerization of the same nucleotide produced a long chain of nucleotide. Arrange many these molecules in the right order produced the first pre-biotic molecule called RNA (Ribonucleic acid) which carried two important instructions to store information and to catalyze the chemical reaction to create the first living cell. A more stable form of the information molecule called DNA (deoxyribonucleic acid by deleting a single Oxygen atom from its ribose sugar molecule) was formed later to store information. The information molecules, DNA, RNA, and protein organized to create the first living cell. Since all living creatures carry the same information molecules, all living creatures must have evolved from that single cell. Life must have begun as an RNS world. Now, we know that life begins with a single cell. Every cell in living creatures contains a nucleus, inside the nucleus we have chromosomes which contain genes and genes are made of DNA, the blueprint of life. The same DNA molecule is responsible for creating all living forms. It was also produced nearly four billion years ago.

Let me give you a very simple analogy; compare your house with a cell. A single cell is so small we cannot see with our naked eye. Using Electron Microscope, we magnify a single fertilized egg to a million times its size, almost the size of a house. This is what we saw inside the cell. A single cell is like your house. Each house has a kitchen; the cell has a nucleus. Imagine your kitchen has 46 volumes of cookbooks; our cell has 46 chromosomes, 23 chromosomes from each parent. Suppose the cookbooks in your kitchen contain 24,000 recipes to make different dishes for your breakfast, lunch and dinner. The chromosomes in our cell contain 24,000 genes which contain instructions to make proteins essential to carry out our body functions. Suppose the cookbooks in your kitchen are written in English, it uses all 26 alphabets in the English language. What is surprising is that the book of life uses only four letters AT, and GC and they come in pairs. These are the initials of four chemicals found in the nucleus of all living cells called nucleotide. As I mentioned earlier, they are Adenine, Thymine, Guanine and Cytosine or AT/GC and their multiple units or polymers known as DNA (Deoxyribonucleic acid.) Every set of three letters of the four genetic letters, codes for a single amino acid and this three-letter code is called Codon. Out of four genetic letters, we get 64 combinations of the three letter codes. This Genetic Code was broken by Marshall Nierenberg in our Lab at NIH for which he was honored with a Noble Prize.

As I said above, Ross was the first person who used Nitrogen Mustard, a chemical weapon used as a chemical weapon during WWI, to attack DNA for preventing cancer growth. Radiolabeled study showed that Nitrogen Mustard shut off a gene by cross-linking both strands of DNA that we inherit one strand from each parent. It was the same Cross-linking agents such as Nitrogen mustard made by Haber. Solders exposed to

Nitrogen Mustard showed a sharp decline of White Blood Cells (WBC) from 5000 cell/CC to 500/CC. Children suffering from Childhood Leukemia have a very high WBC count (over 90,000/CC). Most of the WBCs are premature, defected, and unable to defend the body from microbial infections. Ross rationale was that cancer cells divide faster than the normal cell; by using Nitrogen Mustard he could use cross linking DNA and prevent cell division. Once he demonstrated that he could shut off a gene by cross-linking DNA; he could shut off any mutated gene including the genes of all 220 tissues present in a human by finding a dye that could specifically color that tissue. He could attach the Nitrogen Mustard group to the dye and attack the cancer genes in any one those 220 tissues.

Although Nitrogen Mustard is highly toxic, Ross thought more cancer cell would be destroyed than the normal cells. Over decades, Ross made several hundred derivatives of Nitrogen Mustard as cross-linking agents. Some of the Nitrogen Mustards are useful analog such as Chlorambucil used for treating childhood leukemia (which brought the WBC level down to 5,000/CC) and Melphalan and Myrophine for treating Pharyngeal Carcinomas. Because of the high toxicity of Nitrogen Mustard, new drugs could not be developed to treat other types of Orals or Lung Cancers. [7-13].

As I said above, Ross was the first person to use war chemicals successfully to treat cancer. Although such drugs are highly toxic, more cancer cell will be destroyed than the normal cells. Over decades, Ross made several hundred derivatives of Nitrogen Mustard as cross-linking agents. Some of the Nitrogen Mustards are useful analog such as Chlorambucil used for treating childhood leukemia (which brought the WBC level down to 5,000/CC) and Melphalan and Myrophine for treating Pharyngeal Carcinomas. Because of the high toxicity of Nitrogen Mustard, new drugs could not be developed to treat other types of Orals or Lung Cancers.

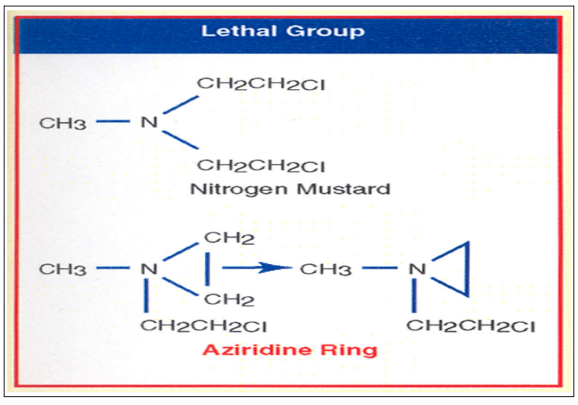
Toxic Effect of Nitrogen Mustard

As his Assistant, I had made several dozens of previously synthesized analogs of Nitrogen Mustards for Professor Ross. I will describe how to make the Nitrogen Mustard by using Haber's crudest method. Haber reacted Methylamine with Ethylene oxide to make 2-bis dihydroxy ethyl methyl amine. It was chlorinated by heating Phosphorus Penta Chloride in Phosphoric Acid. If you noticed a faint smell of Mustard Seed, Congratulations, you got Nitrogen Mustard; you cooled the solution and diluted with ice cold water, the oil floating in the aqueous solution was extracted with Chloroform. The solution is dried, and Hydrogen chloride gas is passed through to make its solid Hydrogen-Chloride salt of Nitrogen Mustard Hydrogen Chloride salt is separated. No matter how many precautions you take, after the completion of the experiment, if you would take an alcohol swab of working bench or walls, doors, knobs and run a mass spectrum of the alcohol extract, you find a spectrum corresponding to Nitrogen Mustard. If you are exposed to Nitrogen Mustard and cross the threshold level, your WBC drops sharply and the energy providing Mitochondria die and you are most likely to freeze to death even during summer. Someone in the Defense department may make it, now-a-day. Safety committee will not approve this study in the University Research Lab. Your IRB (Institutional Review Board) and the safety committee will reject your proposal; and who will provide the funds for such an expensive study. The drug sensitivity between normal cells to cancer cells give a ratio of toxicity called the Chemotherapeutic Index (CI). The higher the ratio the more toxic chemicals are to cancer cells. When tested against Walker Carcinoma 256 in Rats, most Nitrogen Mustards cross-link both strands of DNA and give a CI of ten.

Drug Design to treat animal cancer

Aziridine Analogs as Anti-Cancer Agents serving as Pro-Drugs

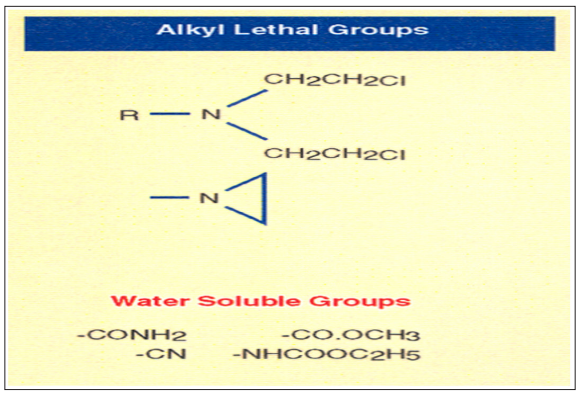
A radiolabel study to understand the mechanism of action of Nitrogen Mustard Ross showed that cross-linking of DNA occurred in two steps. The first step is involved in the formation of a three-member aziridine intermediate which remains stable and inactive in the neutral media (acts as a pro-drug). The second arm of the Nitrogen Mustard generates a highly reactive carbonium ion by enzyme which attacks the first arm of the double stranded DNA. The second arm is attacked, as the cancer cells grow; they use Glucose as a source of energy. Glucose is broken down Lactic Acid. In the presence of acid, the Aziridine ring become activated by generating the carbonium ion which attacks the second arm of the DNA resulting in the cross-linking. This study result showed that cross-linking both strands of DNA is not necessary to shut off a gene, only binding to a single strand of DNA by aziridine could also shut off a gene with half the toxicity. To attack a single strand of DNA, aziridine analogs are separately synthesized. As a part of my doctoral thesis, I was assigned a different path. Instead of cross-linking DNA strands, I am to design drugs to attack only one strand of DNA. The following chart describes the formation of Aziridine ring intermediate. Ross demonstrated that cross=linking both strands of DNA to shut off a gene is not necessary, binding to single strand of DNA by intermediate Aziridine could also shut off a gene. It has a great advantage over Nitrogen Mustard, Aziridine acts as a Pro-drug. The Aziridine ring opens in the acidic medium. The true drug is released a Carbonium ion which attack DNA once the Aziridine opens.



DNA Binding Aziridine Group

This study showed that to attack a single strand of DNA, we must synthesize Aziridine in the Lab by using ethyl amino methyl sulphonate in sodium hydroxide. Pure Aziridine was distilled off. Synthesis of Aziridine analogs will give two advantages over Nitrogen Mustard: first, instead of cross-linking, Aziridine binds to one strand of DNA, reducing its toxicity of the double stranded Nitrogen Mustard by half. Second, it gives selectivity, the Aziridine ring serves as a prodrug. Its ring opens only in the acidic medium. Once the active ingredient Aziridine was

determined to attack DNA, the next question was what drug delivery method should be used to deliver Aziridine at the tumor site.



The above structures are Nitrogen Mustard (2-bischloroethyl methyl amine) and Aziridine.

Designing drugs to bind to a Single Stranded DNA to Treat Animal Cancers

As a part of my doctoral thesis, I was assigned a different path. Instead of cross-linking both strands of DNA by Nitrogen Mustard, I am to design drugs to attack only one strand of DNA by making Aziridine analogues. We decided to use Aziridine moiety (as an intermediate of Nitrogen Mustard) that would be an excellent active component to shut off a gene by binding to a single strand of DNA. To deliver Aziridine to the target site which is the N-7 Guanine of DNA, we decided to use Dinitrophenyl (DNP) moiety as a drug delivery agent. DNP is a dye which colors the tumor tissues of the experimental animal tumor such as Walker Carcinoma 256 in Rats. It is well known that analogs of DNP such as Dinitrophenol disrupt the Oxidative Phosphorylation (OXPHOS) of the ATP (Adenosine Triphosphate) which provides energy to perform all our body functions. To provide energy for our body function, the high energy phosphate bond in ATP is broken down to ADP (Adenosine Diphosphate) which is further broken down to AMP (Adenosine Mono Phosphate), the enzyme Phosphokinase put the inorganic phosphate group back on the AMP giving back the ATP. This cyclic process of Oxidative Phosphorylation is prevented by Dinitrophenol. As a part of my doctoral thesis, I decided to use Dinitrophenol as drug delivery method for the active ingredient aziridine. The analog of DNP such as Aziridine Dinitrophenol could also serves as a dye which stains Walker Carcinoma 256, a solid and most aggressive tumor in Rat. The first compound I made by attaching the C-14 radiolabeled Aziridine to the DNP dye. The Dinitrophenyl Aziridine was synthesized using Dinitrochlorobenzene with C-14 radiolabeled Aziridine in the presence of Triethyl amine which removes the Hydrochloric Acid produced during the reaction. When the compound Dinitrophenyl Aziridine was tested against the implanted experimental animal tumor, the Walker Carcinoma 256 in Rats, it showed a TI (Therapeutic Index) of ten. The TI of ten was like most of the analogs of Nitrogen Mustard. Since this Aziridine analog was not superior to Nitrogen Mustard, it was dismissed as unimportant.

On further reexamination of the X-ray photographs of Dinitrophenyl Aziridine, it appeared that most of the radioactivity was concentrated at the injection site. Very little radioactivity was observed at the tumor site. It was obvious that we need to make derivatives of Dinitrophenyl Aziridine to move the drug from the injection site to the tumor site. Because of the lack of fat/water solubility to be effective drug delivery method, Dinitrophenyl Aziridine stays at the injection site, a very small amount of radioactivity was found on the tumor site.

Designing novel drugs is expensive and time-consuming business. For example, first, you test the drug on experimental animal tumor. The Walker Carcinoma 256 in Rats, an aggressive solid tumor. You determine the structure/activity relationship. The process of developing and approving new drugs and treatment for cancer is rigorous.

Structure-Activity Relationship

I immediately realized that by altering structure, I could enhance biological activity by making water and fat-soluble analogs of Dinitrophenyl Aziridine. By attaching water soluble groups, I should be able to move the drug from the injection site to the tumor site. To deliver 2,4-Dinitrophenylaziridine form the injection site to tumor site, I could alter the structure of 2,4-Dinitrophenylaziridine by introducing the most water-soluble group such as ethyl ester to the least water-soluble group such as Cyano- group or to introduce an intermediate fat/water soluble such as Amido group.

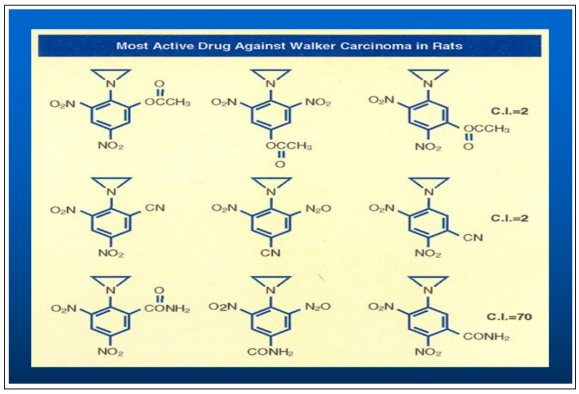
An additional substituent in the Dinitrophenyl Aziridine could give three isomers, Ortho, Meta, and Para substituent. Here confirmational chemistry plays an important role in drug delivery methods. Ortho substituent always gives inactive drug. Model building showed that because of the steric hinderance, Aziridine could not bind to DNA shutting off the genes. On the other hand, Meta and Para substituents offer no steric hindrance and drug could be delivered to DNA. When injected in Rat, because of the high solubility, most of the drugs was pass down through urine and extracted the drug from Rat urine by chloroform, The following chart showed that I synthesized all nine C-14 radiolabeled analogs of 2,4-Dinitrophenyl aziridines and tested them against implanted Walker Carcinoma 256 in Rats.

Derivatization of Dinitro phenyl Benzamide based on Partition Coefficient

The most water-soluble substituent

The first three compounds on top line of the above chart carry all three isomers of the most water-soluble Ethyl Ester group attached to 2,4-Dinitropehny aziridine. The compound in vivo is hydrolyzed Ethyl Ester to produce most water-soluble carboxylic

group. Since it is the most water-soluble substituent, within 24 hours of injection in Rats, the entire radioactive compound was passed down from in the Rat urine, and it can be extracted by Chloroform. Since the Ortho position was not available for DNA binding, it showed no biological activity, but the third compound in which Ortho position was free to bind to DNA showed some anti-tumor activity in Rats.



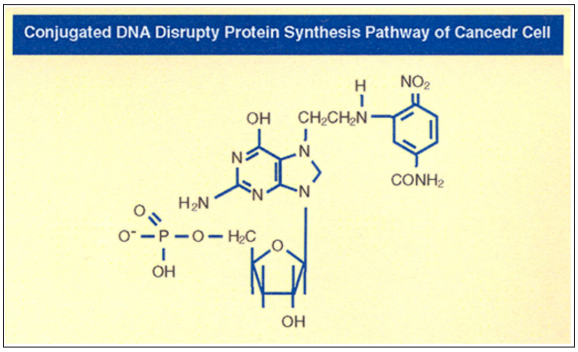
The Least Water-Soluble Substituent

On the other hand, when the least water-soluble Cyano-group was attached to all three isomers of the 2,4-Dinitrophenyl aziridine compound as shown in the second line of the above chart, most of the compound stayed at the injection site. Only the last Cyano-derivative attached to DNA showed some anti-tumor activity.

The Moderately Soluble Amido-Substituent

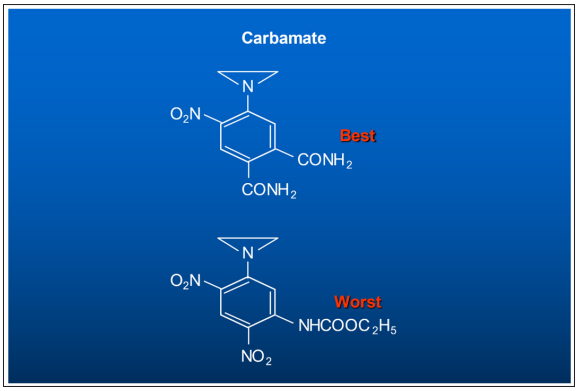
The last line of the above chart showed that the first two Amido groups were sterically hindered and did not bind to DNA and showed no biological activity, but the last compound presents the perfect drug delivery method. The entire drug was delivered from the injection site to the tumor site. The drug 1-Aziridine, 2,4-dinitro, 5-benzamide (CB1954) showed the highest anti-tumor activity. It has a CI of seventy; it is seventy times more toxic to cancer cells; highest toxicity ever recorded against Walker Carcinoma 256 in Rats [14-16].

As I said above, Nitrogen Mustards are highly toxic because they have neither specificity nor selectivity. They attack all dividing cells whether they are normal or abnormal. On the other hand, the analogs of Aziridines and Carbamates serve as prodrugs and remain inactive in the basic and neutral media. They become activated only in the presence of acid produced by growing cancer cells. Aziridine attacks DNA in acidic medium, particularly the N-7 Guanine. The dye Dinitro benzamide has great affinity for Walker Tumor. The Aziridine Dinitro benzamide (CB1954) has the highest toxicity to Walker Tumor cells ever recorded. As the tumor grows, it uses Glucose as a source of energy. Glucose is broken down to Lactic Acid. It is the acid which activates the Aziridine ring. The ring opens to generate a carbonium ion which attacks the most negatively charged N-7 Guanine of DNA (as shown below) shutting off the Walker Carcinoma gene in Rat. The following conjugate structure shows how CB1954 binds to a single strand of DNA shutting off the gene.



Conjugated DNA Disrupting Protein Synthesis Pathway of Cancer Cell

For the discovery of CB1954, The University of London, honored me with the Institute of Cancer Research (ICR) post-doctoral fellowship award to synthesize more analogs of CB1954. Over the years, I made over a hundred additional analogs of Dinitro phenyl aziridines. To increase the toxicity of CB1954 to Walker Carcinoma, I made additional 20 analogs as a postdoctoral fellow. When I attached one more Carbonium ion generating moiety, the Carbamate moiety to the Aziridine Dinitrobenzene, the compound Aziridine Dinitro benzamide Carbamate was deadliest. It was so toxic that its Therapeutic Index could not be measured. We stopped the work. Further work at London University was discontinued for safety reasons.



The Best and the Worst Dinitro phenyl Aziridine Analogs

Although Aziridine Carbamate is extremely toxic, it is also very useful in testing the sensitivity of tumors in Tumor Bank. Over the years, some tumors in the tumor bank could become resistant. If a tumor culture survives in a petri dish by adding a solution of Aziridine Dinitrobenzene Carbamate, it means that this tumor has become resistant over the years and must be replaced by new sensitive tumor cells. The combination of Aziridine and Carbamate is the deadliest to cells.

In developing drugs for treatments, we poison bad DNA selectively. All poisons are a class of chemicals that attack all DNA good and bad alike. Chemicals that cause cancer, at a safe level, can also cure cancer. Science teaches us to selectively attack bad sets of DNAs without harming the good sets of DNAs. Poisons are injurious to living creatures. There is a small class of chemicals, when exposed to humans, disrupt the function of DNAs, and make normal cells abnormal and they are called cancer causing chemicals or carcinogens. I must confess, we still use surgery to cut off cancerous breasts; we still burn cancer cells by radiation; and we still poison cancer cells by

chemicals. The largest killer of women is breast cancer. After all the treatment, the remaining cancer cells return as metastatic cells and kill breast cancer patients in three years. A decade from now, these methods could be considered brutal and savage, but today that is all we have. We hope to develop new treatment for Breast Cancer. Hope means never ever giving up.

Cryopreservation

Cryopreservation Labs freeze eggs for years below seventy degrees. Human eggs are located on X-chromosome. It is a long chromosome and is made of 164 million AT-GC base pairs and carry 1,144 genes. On the other hand, Y-chromosome located on the sperm is much smaller than X-chromosome. It is made of 59 million AT-GC base pairs and carries 255 genes. We have no idea how many of those genes present on either X & Y-chromosomes carry deleterious mutation. Freezing X & Y chromosomes in the Cryo-Lab for later use is a bad idea. Instead, I recommend that Cryopreservation Labs extend their responsibility to include Sequencing Lab to sequence the X&Y chromosomes and discard any egg or sperm which carry deleterious mutations. Only deleterious mutation free egg and sperm should be fertilized in vitro. As fertilized egg becomes an 8-cell embryo within three days of incubation. Each cell must be sequenced separately. Only those cells that are free from any deleterious mutation should be cryopreserved for future use. To have healthy baby, young couples should conceive at early age and cryopreserve the deleterious mutation free cell for future use. After years of cryopreservation, when the couple decided to have a baby, the cell must be sequenced once again to ensure that it has not acquired any deleterious mutation.

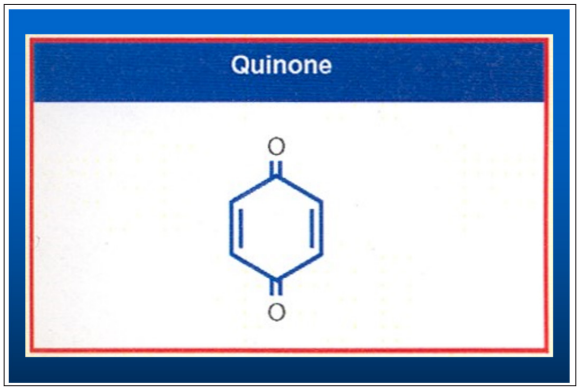
Drug Design to Treat Human Cancer

The Rational for Designing drugs to treat Glioblastoma, Human brain cancers

I design drugs to treat Glioblastoma the brain cancer. In the pre-genome era, if the sequence in the Brain tumor confirms the presence of a mutation in the SNP responsible for causing the Glioblastoma, since there was no treatment, the patient dies within fourteen months of diagnosis. Glioblastoma is a solid aggressive tumor. It grows so rapidly, it crushes the neuronal circuits, it cushes the synapses and most patients internally bleed to death within fourteen months.

Glioblastoma (GBM) is a primary type of brain cancer which originates in the brain, rather than traveling to the brain from other parts of the body, such as the lungs or breasts. GBM is also called glioblastoma multiforme which is the most common type of primary brain cancer in adult humans. Attaching Nitrogen Mustard group to a carrier dye will produce highly toxic compound which will have neither specificity nor selectivity. Such a compound will attack all dividing cells whether they are normal or abnormal. On the other hand, the analogs of Aziridines and Carbamates serve as prodrugs that are that they remain inactive in the basic and neutral media. They become activated and produce powerful carbonium ions which attack DNA only in the presence of acid produced by cancer cells.

One day, I heard an afternoon lecture at the NIH in which the speaker described that radio labeled Methylated Quinone crosses the Blood Brain Barrier (BBB) in mice. When injected in mice, the X-ray photograph showed that the entire radioactivity was concentrated in the Mice's brain within 24 hours. I immediately realized that Glioblastoma multiforme, the brain tumor in humans, is a solid aggressive tumor like Walker Carcinoma in Rats. I decided to use Quinone moiety as a novel drug delivery molecule to cross BBB (Blood Brain Barrier) delivering Aziridine rings to attack Glioblastomas. By introducing an additional Carbamate moiety, I could increase its toxicity several folds. I planned to use this rationale to translate animal work to human by introducing multiple Aziridine and Carbamate moieties to the Quinone molecule to test against Glioblastomas in humans.



The Structure of a non-toxic and non-addictive Quinone used for crossing the Blood Brain Barrier (BBB)

With the Quinone ring, I could introduce two Aziridine rings and two Carbamate moieties and could create havoc for Glioblastoma. Within three years, I made 45 analogs of Quinone. One of the Quinone carries two aziridines and two carbamate moieties which was highly toxic to Glioblastoma. The tumor stopped growing and started shrinking. I named the Di-aziridine Dicarbamate Quinone, AZQ. My major concern was how toxic this compound would be to normal brain cells. Fortunately, brain cells do not divide, only cancer cells divide. AZQ acts as a Prodrug. A Prodrug is compound carrying a chemical by masking group that renders it inactive and nontoxic. Once the prodrug reaches a treatment site in the body, removing the mask frees the active drug to go only where it is needed, which helps avoid systemic side effects. Aziridine and Carbamate show selectivity. As I said above, to grow rapidly, cancer cells use Glucose as a source of energy. Glucose is broken down to produce Lactic acid. It is the acid which activates the prodrug aziridine and carbamate moieties generating Carbonium ions attacking Glioblastoma which stops growing and start shrinking.

My drug AZQ is successful in treating experimental brain tumors because I rationally designed to attacks dividing DNA. Radio labeled studies showed that AZQ bind to the cancer cells DNA and destroy brain tumor and normal brain cells are not affected at all. AZQ is a new generation of drugs. Not so long ago, brain cancers mean death. Now, we have changed it from certain death to certain survival. The immunologists in our laboratories are developing new treatment techniques by making radio labeled antigens to attack remaining cancer cells without harming normal cells.

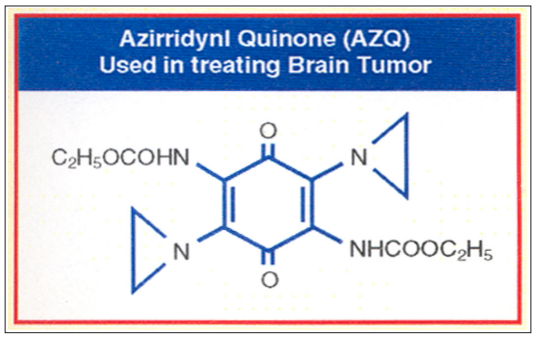
We have cured many forms of cancer. We have eliminated childhood leukemia, Hodgkin disease, testicular cancer and now

AZQ type compounds which are being developed rationally. While most anti-cancer drugs such as Adriamycin, Mitomycin C, Bleomycin etc., in the market are selected after a random trial of thousands of chemicals by NCI, AZQ is rationally designed for attacking the DNA of cancer cells in the brain without harming the normal cells. We are testing combinations of these drugs to treat a variety of experimental cancers in animals. [17,18].

Single strand DNA Binding Aziridines & Carbamate

From the studies of Dinitrophenyl analogs on Wlaker Carcinoma-256 Rat, I learned that the combination of Aziridine and Carbamate confer the highest toxicity to the tumor cells.

I decided to use Quinone as a carrier for Aziridine and Carbamate moieties to attack Glioblastomas. By introducing additional Carbamate and Aziridine moieties, I could increase its toxicity several folds. I planned to use this rational to translate animal work to human by introducing multiple Aziridine and Carbamate moieties to the Quinone to test against Glioblastomas in humans. Over the years, I made dozens of analogs of Aziridine Quinone. By attaching two Aziridines and two Carbamate moieties to Quinone, I synthesized the most useful compound, Diaziridine Dicarbamate Quinone, I named this novel compound AZQ. Over three-year period, I made 45 analogs of AZQ. They were all considered valuable enough to be patented by the US Government (US Patent 4,233,215). By treating brain cancer with AZQ, we observed that Glioblastoma tumor not only stops growing, but it also starts shrinking. I could take care of at least one form of deadliest old age cancer, Glioblastomas. Literature search showed that AZQ is extensively studied as a pure drug and in combination with other anti-cancer drugs [19,20].



U.S. Patent 4,146,622

Single Strand DNA Binding Aziridine and Carbamate

As I said above, Glioblastomas, brain cancer, is a solid and aggressive tumor and is caused by mutations on several sites in chromosomal DNA. Deleterious genetic mutations are the result of damaging DNA nucleotides by exposure to radiation, chemical and environmental pollution, viral infections, or genetic inheritance. The other factors responsible for causing DNA mutations are due to the fast rate of replication of DNA. For example, the bacteria E-coli grows so rapidly that within 24 hours, a single cell on a petri dish containing nutrients forms an entire colony of millions when incubated on the Agar Gel. Mistakes occur in DNA during rapidly replication such as Insertion of a piece of DNA, Deletion, Inversion, Trans location, Multiple Copying, Homologous Recombination etc. When an additional piece of nucleotide is attached to a DNA string, it is called Insertion, or a piece of DNA is removed from the DNA string; it is called Deletion or structural Inversion of DNA is also responsible for mutations. Since the gene codes for Proteins, Insertion and Deletion on DNA have catastrophic effects on protein synthesis. With the Quinone ring as a carrier across BBB, I could introduce different combinations of Aziridine rings and Carbamate moieties to Quinine and could create havoc for Glioblastomas. My major concern was how toxic this compound would be to the human brain cells. Fortunately, brain cells do not divide, only cancer cells divide. Attempting to find the site of mutations on Glioblastomas represents the greatest challenge. In Glioblastomas, three major changes occur on Chromosomes (C-7, C-9 & C-10) and two minor changes occur on Chromosomes (C-1 & C-19). These mutations are responsible for causing brain cancers in humans. Let us examine the effect on each chromosome. In a normal human cell, Chromosome-7 which is made of 171 million nucleotide base pairs, and it carries 1,378 genes. When Insertion occurs on Chromosome-7. Ninety-seven percent of Glioblastoma patients are affected by this mutation. On the other hand, a different mutation occurs on Chromosome-9 which is made of 145 million nucleotide base pairs, and it carries 1,076 genes. A major Deletion of a piece of DNA occurs on Chromosome-9 which results in eighty- three percent patients who are affected by this mutation. A minor Deletion of DNA also occurs on Chromosome-10 which is made of 144 million base pairs, and it carries 923 genes. Although it is a minor deletion of a piece of DNA and yet it contributes to ninety-one percent patients with Glioblastoma. To a lesser extent, small mutation occurs on Chromosome-1 (the largest Chromosome in our Genome). It is made of 263 million nucleotide base pairs and carries 2,610 genes) and Chromosome-19 (it is made of 67 million base pairs and carries 1,592 genes) are also implicated in some forms of Glioblastomas.

All known Glioblastomas causing genes are located on five different chromosomes and carry a total of 9,579 genes. It appears impossible to design drugs to treat Glioblastomas since we do not know which nucleotide on which gene and on which chromosome is responsible for causing the disease. It becomes possible by using C-14 radiolabeled Aziridines, we can confirm the binding site of a nucleotide on a specific gene and on a specific chromosome. By comparing with the Reference Sequence described above. We can further confirm the sites of mutations.

With the completion of 1,000 Human Genome Project, it becomes easier. by simply comparing the patient's genome with the sequence of 1000-genomes, letter by letter, word by word and sentence by sentence, we could identify the differences called the variants with precision and accuracy, the exact variants, or mutations responsible for causing the disease. Once the diagnosis is confirmed, the next step is how to treat the disease. As I explained above, by making CB 1954 to treat the solid Walker Carcinoma in Rats, I established the structure activity relationship. I also demonstrated that by adding Aziridine and Carbamate to the dinitro-phenyl moieties, I could make the most toxic compound. Since Quinone crosses the Blood Brain Barries. I decided to attach these toxic moieties to Quinone making AZQ to treat human Glioblastoma. Since these moieties serve as Pro-drug, they become active drug in acidic medium. Tumors grow faster than normal cells; they use glucose as a source of energy.

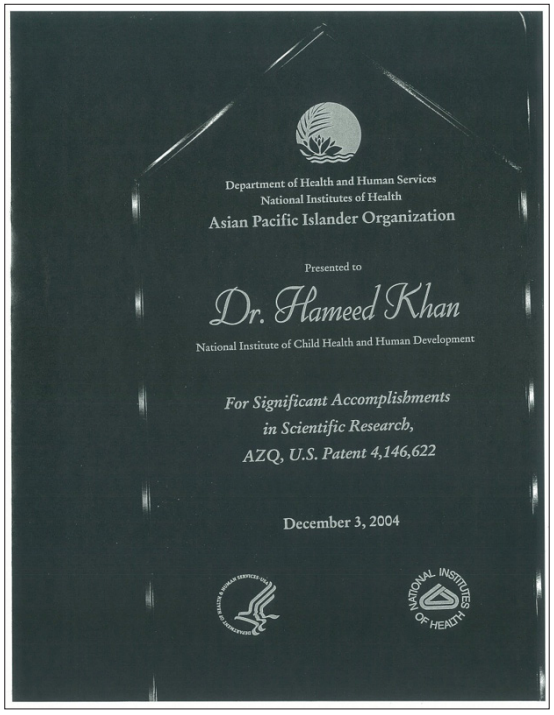
Glucose breaks down to produce lactic acid. It is acid which breaks down the prodrug and releases the active drug attacking the tumor. We have demonstrated that all bad genes can be shut off using Aziridine or Carbamate or both as attacking the tumors shutting off the genes. If you plan to develop drugs to treat other cancers, all we need to do is to identify carriers such as coloring dyes which stains a specific tumor. By attaching Aziridines and Carbamate moiety to carriers' dyes, we could attack other tumors.

One of the greatest challenges of nanotechnology is to seek out the very first abnormal cell in the presence of billions of normal cells of our brain and shut off the genes before it spread. I worked on this assignment for about a quarter of a century; conducted over 500 experiments which resulted in 200 novel drugs. They were all tested against experimental animal tumors. Forty-five of them were considered valuable enough to be patented by the US Government (US Patent 4, 146, 622 & 4,233,215). One of them is AZQ which not only stops the growth of Glioblastoma, but also the tumor starts shrinking. For the discovery of AZQ, I was honored with, "The 2004 NIH Scientific Achievement Award." One of America's highest Award in Medicine. I was also honored with the India's National Medal of Honor, "Vidya Ratna" a Gold Medal. (see Exhibits 1,2,3,4)

Exhibit # 1

2004 NIH Scientific Achievement Award
Presented to
Dr. Hameed Khan
By
Dr. Elias Zerhouni,
The Director of NIH
During the NIH/APAO Award Ceremony held on December 3, 2004.

Dr. Khan is the Discoverer of AZQ (US Patent 4,146,622 & 4,233,215), a Novel Experimental Drug Specifically Designed to shut off a Gene that causes Brain Cancer for which he receives a 17-year Royalty for his invention (License Number L-0I9-0I/0). To this date, more than 300 research papers have been published on AZQ. The award ceremony was broadcast live worldwide by the Voice of America (VOA). Dr. Khan is the first Indian to receive one of America's highest awards in Medicine.



2004 NIH Scientific Achievement Award

Exhibit # 2

His Excellency, Dr. A.P.J. Abdul Kalam,
The President of India
Greeting
Dr. A. Hameed Khan,

Discoverer of anti-cancer AZQ, after receiving 2004, Vaidya Ratna,
The Gold Medal, One Of India's Highest Awards in Medicine At The Rashtrapathi Bhavan (Presidential Palace), in Delhi, India, During a Reception held on April 2, 2004.

Exhibit # 3

Dr. Khan was invited to give Maharaja Theromal Memorial Lecture. After the lecture, the Royal Family invited Dr. and Mrs. Khan for tea at the Royal Palace.

The Royals of Travancore

Dr. Hameed Khan of NIH was invited to give the "Maharaja Thrumal Memorial Award Lecture" "On the Impact of Genetic Revolution on our lives during 21st Century and Beyond" at the University of Trevandrum. After the lecture, His Royal Highness Sree Padmanabha Dasa Marthanda Varma (the brother-in-law) of Her Royal Highness Maharani Travancore (on his left) invited Dr. Hameed Khan and Mrs. Vijayalakshmi Khan for the Tea at the Pattom Palace at Thiruvanthapuram on May 12, 1999. Standing on Dr. Khan's right is the Son-in law of Her Royal Highness, the Maharani.

Exhibit # 4

Gold Medal for Dr. Khan

Dr. A. Hameed Khan, a Scientist at the National Institutes of Health (NIH) USA, an American Scientist of Indian Origin was awarded on April 2, 2004. Vaidya Ratna; The gold Medal, one of India's Highest Awards in Medicine for his Discovery of AZQ (US Patent 4,146,622) which is now undergoing Clinical Trials for Treating Bran Cancer.

Glioblastoma (GBM) has no cure because of its aggressive, infiltrative growth pattern, extreme cellular diversity, and location behind the blood-brain barrier. These factors allow the tumor to aggressively evade standard treatments, such as surgery and chemotherapy, causing rapid regrowth. AZQ does not cure Glioblastoma. It simply shrinks the tumor. Most Glioblastoma patients die within fourteen months of their diagnosis. After the treatment with AZQ life of Glioblastoma patients may extend to two additional years. Sequencing the single cell and comparing it with Reference Sequence from the embryo will not help because too many mutated genes are involved. Identified mutations included CHEK2 (c.1477G>A, p.E493K), IDH1 (c.395G>A, p.R132H), and TP53 mutations. Non-coding regions exhibited variants in the TERT promoter (c.-146C>T, c.-124C>T) and TP53 RNA splicing site (c.376-2A>C, c.376-2A>G).

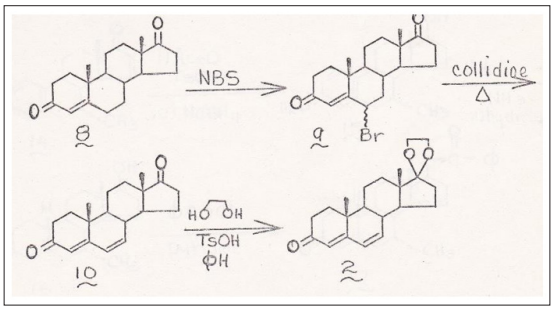
It may be that AZQ is shutting few genes not all. To shut off other genes, we must continue to develop more effective drugs. It is up to the Manufacturer of AZQ to take the next to conduct further studies and submit the safety data of AZQ to FDA for approval.

Drug Approval Process

An oversimplified picture of the New Drug approval process is described below. For more accurate information, check CFR. The manufacturer conducts a 90-toxicity study to determine a safe level followed by a 2-year chromonic study to see that it is neither a carcinogen nor allergen. The data is submitted to the Food & Drug Administration (FDA). Scientists at FDA follow the rules described in CFR (Code for Regulation) by setting up an expert review committee. Members of the committee could recommend additional safety tests If they are satisfied, FDA issues a, IND (Investigational New Drug). When IND is approved, it permits clinical trial. In Phase-I, 30 patients are treated. If it is safe, Phase-II is recommended in which 300 patients are tested. If no adverse effects are observed, the drug is tested in Phase-III screening in which 3,000 patients are tested. Even after the approval of Phase-III testing, a yearly Adverse Reaction report is required. If Adverse Reaction is observed, the drug is withdrawn. If safe an NADA, new drug approval issued. The drug will go to clinical trials. Here is an example from my doctoral thesis.

How to Design Drugs to Treat Metastatic Breast Cancer

Recent, Radiolabeled studies in mice showed that male hormone Testosterone has great affinity for female organs like Breast, Ovary, and Fallopian tube cells. On the other hand, Estrogen, the female hormone, has great affinity for male Prostate gland. By attaching multiple Aziridine rings and Carbamate ions to both Hormones, I could design novel drugs to attack both the Breast and the Prostate cancers. Now, I found that I could increase its toxicity several folds to abnormal cells by attaching more than four Aziridine and Carbamate moieties to both Male and Female Hormones. In a Breast tumor, within the start and stop codon, BRCA1 gene has captured over two hundred thousand nucleotide bases. The BRCA1 gene carries about three thousand mutations. These mutations are caused by exposure to radiation, chemical or environmental pollutants, viral infection or genetic inheritance. To attack the mutated nucleotides among the three thousand mutations in BRCA1 gene, we could use male hormone, Testosterone, and bind multiple radio-labeled Aziridine and Carbamate ions to attack BRCA1 mutations. By using three-dimensional MRI [19], we could show how many radio-labeled nucleotides were bound to which mutations. Out of seventeen positions available for substitutions on Testosterone ring system. There are only three positions that are 1,3 and 17 are available for substitution on Testosterone ring system.



Carl Djerassi had demonstrated that we could activate additional positions for substitutions on hormone ring system such as the position 9 and 10 by reacting with Bromo-acetamide which introduce a Bromo ion on position 10 which could be de-brominated by Collidine to introduce a 9,10 double bond which we could further brominate to produce 9,10 dibromo compound. These bromo ion could be replaced by additional Aziridines or Carbamate ions. We could increase or decrease the number of Aziridine and Carbamate ions to get maximum benefit by further brominating position 15 and 16 to introduce additional Aziridine and Carbamate moieties.

Similarly, we could use the female hormone Estrogen and by attaching multiple Aziridine and Carbamate ions to attack Prostate tumor in Men. Since there are seventeen positions also available on Estrogen ring as well; again, we could increase or decrease the number of Aziridine and Carbamate ions to get the maximum benefit by using Djerassi' method as we did with Testosterone. The above methods are novel approach to designing drugs to treat Breast and Prostate cancers using genetic make-up of a patient to treat metastatic cancers.

Conclusion

In pre-genome era, women have children by chance. All childbearing women should have asked themselves that the child they are bringing to this world is acceptable member of the humane society. In today's world women have a choice to have children either by chance or by choice. Before Genome Sequencing, women have no choice, but to have children by chance and took great risk of giving birth to defected children. They are responsible for filling our prisons, mental hospitals and Asylums. After sequencing the human genome, sequencing the embryo's genome and comparing it with the Reference Sequence will identify deleterious mutations. Today, women have a choice to select the very best cell for implantation. They will save themselves and society. As for scientists, designing new drugs to treat patients by introducing multiple Aziridine and Carbamate moieties to male hormone, you are producing the most toxic compounds. The man who made Aziridine JW (James Worwich) died of Aziridine exposure. Aziridine absorbs through the skin and kill the White Blood Cell (WBC). The bone marrow is severely damaged. Because of the lack of WBC, JW freeze to death. Scientists must take risks. As Sir Winston Churchill, the Prime Minister of England and hero of the WII, had said. "Success comes to those who dare and act and it seldom goes to coward who are ever afraid of consequences." Next generation of scientists (My students) must also take risk. America is the greatest country in the world. It is scientifically and technologically most advanced nation. Americans win more Nobel prizes than any country in the world. It is also the most competitive society. To be successful in this country, we need to screen all children before they are born. We need outstanding men and women. They will be the vanguard of Research and technology. We bequeath the future of this great nation in their hands. We know that they will do their best to keep America the sole remaining superpower of Western World, a Jewel in the crown, a beacon of light, and a shining city on the hill.

The ideas expressed in this lecture are mine and do not represent NIH policies.

The Author

Dr. Hameed Khan was born in Hyderabad, India, educated in England and received his doctorate degree in Chemistry from the University of London. He is a recipient of the Institute of the Cancer Research postdoctoral award of the Royal Cancer Hospital, University of London and a recipient of the Fogarty International postdoctoral award of the National Institutes of Health (NIH), and the National Cancer Institute of USA. He is a discoverer of AZQ (US Patent 4,146,622 & 4,233,215)) for which he was honored with the "2004 NIH Scientific Achievement Award", one of America's highest awards in Medicine. He was also honored with a National Medal of Honor, the Gold Medal by the Government of India. He is a Fellow of the American Institute of Chemistry and was elected to the American Science Advisory Board.

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