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The Impact of Diagnostic MRI on the Early Detection of Lethal Genes in Human Genome and to Develop Genomic Medicine to Treat Brain Cancers

A. Hameed Khan, Ph.D.

Senior Scientist, Department of Genetics & Robotics, NCMRR (National Center for Medical Rehabilitation Research), National Institutes of Health (NIH), Bethesda, Maryland.

***Correspondence:**

Hameed Khan A, Senior Scientist, Department of Genetics & Robotics, NCMRR (National Center for Medical Rehabilitation Research), National Institutes of Health (NIH), Bethesda, Maryland.

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ABSTRACT

Early detection of the onset of diseases are essential if we want to maintain good health. MRI technique provides a three-dimensional image of a microscopic lesion in an organ. Human body is made of 220 different tissues which interact to make an organ and several organs interact to make a human. As a part of medical record, taking MRI of each organ every year and comparing them with each other will identify the appearance of microscopic changes. For example, if you are diagnosed with Brain cancer today, you did not get the cancer yesterday. Abnormal changes are the result of accumulation of harmful mutations over the years predicting the onset of diseases. Once the brain tumor is confirmed, the patient dies within fourteen months. To save the life of the patient, the following three strategies are available and they are Surgery, Radiation and Chemotherapy. This article describes the Chemotherapeutic approach to treat cancers in general and brain cancer, Glioblastoma, in particular. Using rational approach, we designed AZQ (US Patent 4,146,622 & 4,233,215) to treat Glioblastomas. MRI Would identify appearance of microscopic lesions of Glioblastoma and help us start treatment with AZQ long before the disease is confirmed. Using similar rational approach of early diagnosis with MRI, we could design drugs to treat other diseases including cancers.

Keywords

MRI, AZQ, DNA, RNA, Genome Sequencing, Epigenetics.

Introduction

To maintain good health, early detection of a disease is very helpful. The difference between Reactive and Predictive medicine is that in Reactive medicine, we start treating a disease after the disease has progressed and is confirmed. For example, in cancer patient early detection of the Primary tumor is of utmost important. The primary tumor will respond to treatment by either radiotherapy, surgery or chemotherapy. If it is not detected early, the tumor progressed to metastasize and the secondary tumor spread to all other organs and make it impossible to treat. MRI (Magnetic Resonance Imaging) determines the stage of the disease in order to diagnose and treat a disease. On the other hand, in Predictive medicine, primary tumor is identified long before it is confirmed by using physical techniques such as X-rays, CT scans or ultrasound. Of all the technique available, MRI is the best. MRI is a scanning technique for creating detailed images of the human body in three dimensions. The scan uses a strong magnetic field and radio waves to generate images of parts of the body that cannot be seen with other techniques such as X-rays, CT scans or ultrasound.

MRI technique is safe and effective in creating clinically useful images and it is safer than X-ray imaging or CT scanner. In X-ray imaging, the technique uses ionizing radiations to create an image. Prolong exposure to X-radiation damage DNA causing mutations resulting in cancers. In MRI, technique images are generated by frequencies alterations. While in X-ray generate two-dimensional images, in MRI techniques, three-dimensional images are generated which provides in vivid details of microscopic changes in cellular structures over time. For comparison, MRI of the same organ taken over years identify minute changes which is the accumulation of mutations over the years predicting the onset of damage resulting in diseases of an organ. Our body is made of 70 % water, which is made of Hydrogen and Oxygen atoms.

MRI relies on the magnetic properties of the Hydrogen atom to produce images. The Hydrogen nucleus is composed of a single electron, a single Proton and no Neutron. The spinning charged particle produces a magnetic field called a magnetic moment. It produces strong magnetic field in three dimensions. MRI could help us identify earliest development of hard-to-treat diseases such as Brain cancers.

Our senses allow us to observe and understand the world around us. There are five main ways we can do this: through Sight (with our eyes), touch (with our fingers), smell (with our nose), taste (with our tongue) and hearing (with our ears). Through our five senses, our Brain receives a billion bits of data each day. The Brain acts like computer as a Central Processing Unit (CPU). Our Genome carries about 24,000 genes of which one-third functions in our Brain. They are expressed through our following five senses:
Eye**:** There are about one hundred million receptors in Retina of the eye. There are two different kinds of photo receptors and they are present in the Eye, a Cone-shaped and a Rod-shaped. The Rod-shaped photo receptors act as a Prism; they split the light into seven different colors. The object you see Red or Blue color because all other colors are absorbed or filtered out except the Red or Blue you see. The White color appears when all color of lights is reflected. When an object appears Black, when all colors are absorbed. This message goes to the central part of our Brain.

Hearing: There are only sixteen thousand hair cells in a human Ear. We always wonder how receptor Neurons in the Ear; the so-called Hair cells responds to sound; how animals use sound to commute as object location in a space? How information about smell is coded in the Brain? How receptor Neurons respond to Light to Vibration in the air to ordain molecules how these messages are coded? Now we know that nearly all sensor signals go first to a relay station in the Thalamus, a central structure in the Brain. The message then travels to primary sensory area in the Cerebral Cortex. A different area for each sense where they are modified and sent to a higher region of the Brain. Somewhere along the way the Brain figures out what the message means. Nevertheless, the signals that a Mosquito has landed on the back of your hand. Your left hand comes through loud and clear in a fraction of a second through a decision process that leads to you to swat the insect at just the right place.

Taste: Our Taste buds provide a whole different sensation, Sweat, Salty, Sour, and Bitter. Other flavors come from smell and when the nose is blocked as by Cold, most food taste bland and tasteless.

Smell: The average human being can recognize some ten thousand separate Odors. We are surrounded by ardent molecules that animates from trees flowers herbs animals' food in the cell activity bacterial decomposition and other humans. Yet when we want to describe these Myriads odors, we often resort to crude analogies something smells like sweat like ammonia. There are five million Olfactory Neurons plus their supporting cells and stem cells hundreds different genes are identified. Unfortunately, these signals from billions of Brain cells prove almost impossible to unscramble. Scientists can now analyze sensory Neurons far more precisely down to the level of the specific gene and protein within these Neurons. As you all know genes contain a set of instructions all written in AT and GC to make protein. These proteins are called Neuropeptides which are released into your circulating system. Our immunological system is our circulating Nervous system. Neuropeptide circulate in our vascular system until it comes in contact with the receptor site and bring the instructed change.

Sensory neurons**: Sensory** neurons are the nerve cells that are activated by sensory input from the environment - for example, when you touch a hot surface with your fingertips, the sensory neurons will be the ones firing and sending off signals to the rest of the nervous system about the information they have received.

Our Brain is made of about one hundred billion cells called Neurons. Messages pass through our Neurons as Electrical Impulses. Each Neuron is linked to another Neuron by one thousand to ten thousand connections called Synapses. At the end of each Neuron, the Electrical signal stops at the Synapses. Chemical messengers called Neurotransmitters carry the messages from one Synapses to other Synapses and they are Epinephrin, Norepinephrine, Adrenalin, Serotonin etc. The second Neuron converts its Chemical messages received from its Synapses to Electrical signals and the information flows to the next Neuron at speed of 400 kilometer per hour. Although we have not found a Master Gene, all messages are concentrated in the CPU, where the information is received, processed and its response is sent out for the appropriate action.

All neurons are sensitive to mutations leading to diseases. Comparing MRI scans of our Brain taken every year as a part of medical record, we could identify the development of microscopic lesions, which are early sign of the development of Glioblastoma, the Brain cancer. Only fourteen percent of our Bain is made of Neurons, the rest matter is made of Glia, the Glu cells. Abnormal mutations at these cells are responsible for causing Glioblastoma. With help of biopsy, we could isolate a small piece of the Glioblastoma lesion for the DNA sequencing.

Once the damaged cell is identified by MRI, we sequence its entire genome to identify the damaged nucleotides and its location on various genes and their chromosomes. After splicing that is removing the non-coding nucleotides, the DNA is transcribed into a single stranded RNA called m-RNA, which carries hundreds of three-letter Codons which codes for specific Amino acids. We identify a gene by its start and stop codons. The start codon of a gene is AUG which codes for amino acid Methionine. There are three stop codons UAG, UGG, and UGA. Once a single stop codon appears, DNA synthesis stops. By comparing the sequence of each mutated gene with the Reference Sequence Genome, we can identify the location and accumulation of mutations responsible for causing the Glioblastoma.

In 1990, US Congress authorized three billion dollars to our Labs in NIH (National Institutes of Health – an agency of US

Government) to decipher the entire Human Genome to map, identify and locate all genes under the title, "The Human Genome Project." We found that our genome contains six billion four hundred million nucleotides bases half comes from our father and another half comes from our mother. Less than two percent of our Genome contains genes, which code for proteins. The other 98 percent of our genome contains switches, promoters, terminators etc. The 46 Chromosomes present in each cell of our body are the greatest library of the Human Book of Life on planet Earth. The Chromosomes carry genes, which are written in nucleotides. Before sequencing (determining the number and the order of the four nucleotides on a Chromosomes), it is essential to know how many genes are present on each Chromosome in our Genome. The Human Genome Project has identified not only the number of nucleotides on each Chromosome, but also the number of genes on each chromosome.

Our entire genome, the book of our life, is written in four nucleotides. As stated above, they are A, (Adenine) T (Thiamine), G (Guanine) and C (Cytosine). The chain of these nucleotides forms a double stranded string of nucleotides, one strand is inherited from our mother and another from our father, running in opposite directions called the DNA (Deoxy Ribonucleic Acid). According to Francis Crick's Central Dogma of Molecular Biology, [1] double stranded DNA is transcribed into a single stranded RNA (Ribonucleic Acid) which is translated in the Ribosome into proteins. The discovery of the double helical structure of DNA explained how the information to create life is stored, replicate, evolved and passed on to the next generation. This discovery opened a new world order of ideas and buried the old explanation of the magical mystical appearance of life on Earth.

The double stranded DNA explained that the essence of life is information and the information is located on these four nucleotides. Every set of three nucleotide on the mRNA forms a codon, which codes for a specific amino acid. The four-letter text of nucleotides forms a three letter Codon which codes for an amino acid. There are 64 different combinations of Codons, which codes for all 20 amino acids. Sequencing human genome identifies the number of nucleotides and the order *i*n which they are arranged. Less than two percent of our genome contains regulatory region, a piece of DNA, which controls the function of genes. More than 300 regulatory regions have been identified. More than ninety-eight percent of our Genome contains non-coding region used to be called the Junk DNA which makes up to sixty percent of our entire Genome. The non-coding regions contains repetitive piece of DNA, which is tightly packed, and mostly remain silent. The sequencing of this region showed that the non-coding region is the part of Viruses and Bacteria picked up by our Genome during the millions of years of our evolutionary process. During Bacterial or Viral infection, the non-coding DNA could unfold transcribing into RNA resulting into hazardous protein, which could create havoc for our health.

To obtain the Reference Sequence, we have completely sequenced the entire human genome for comparison. All one hundred billion Neurons contain Nucleus. Each Nucleus carries total genetic information to make us that is the Human genome. We are the loving union of our parents. We inherit half of our Genome from our Farther and another half from our Mother. Each Genome is made of six billion four hundred million Nucleotides. [2-6].

The following list provides the details composition of each Chromosome including the number of nucleotides and the number of genes on each Chromosome:

We found that the Chromosome-1 is the largest Chromosome carrying 263 million A, T, G and C nucleotides 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. Chromosome-22 contains 56 million nucleotides and carries 701 genes. Finally, the sex chromosome of all female called the (X) contains 164 million nucleotide bases and carries 1,141 genes. The male sperm chromosome 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. As I said above, a gene codes for a protein, not all 24,000 genes code for proteins. It is estimated that less than 19,000 genes code for protein. Because of the alternative splicing, each gene codes for more than one allele and each allele codes for a different amino acid. All functional genes in our body make less than 50,000 proteins, which interact in millions of different ways to give a single cell. Millions of cells interact to give a tissue, hundreds of tissues interact to give an organ and several organs interact to make a human. In normal humans, the above Genome works perfectly.

Epigenetic answers one the most important questions in the cellular evolution. We all carry 220 different tissues in our body and yet we have a single genome that is the same DNA in every cell. How can all cells carry 24,000 genes and have the same DNA made of AT and GC nucleotides and yet they function in all 220 different tissues? The answer is not all-24,000 genes function in every cell of our body at the same time. Small fraction of genes function in different organs and the rest are turned off by either Methylation or Acetylation, which serves as Epigenetics agents. For Methylation and Acetylation, the common reagent in the Lab is Dimethyl sulphate or Diazomethane in Sodium Hydroxide for Methylation and Acetic Anhydride in Sulfuric Acid for Acetylation. The common Epigenetic agents in our body are Folic Acid responsible for Methylation and Acetyl Choline acts as Acetylating Agents. They can Alkylate or Acetylate both DNA and Histone proteins shutting off genes either temporarily or permanently.

Methylation is a common and widely used mechanism for Epigenetic modifications in cells. Abnormal mutations in the Epigenome have been shown to be correlated with many human diseases, including different cancers, autoimmune disorders, neurological disorders (Fragile X syndrome as well as Huntington, Alzheimer, and Parkinson diseases including schizophrenia).

MRI taken yearly could identify abnormal changes called mutations occurring in our Genome when it is exposed to radiations, or chemical environmental pollution, viral infections or genetic inheritance. Abnormal changes are the result of accumulation of harmful mutations which damage the codons producing wrong protein and the wrong protein is responsible for creating havoc in our body by disrupting biological pathways, destroying DNA repair process, producing uncontrollable cell growth, invading neighboring tissues, disabling normal gene functions, spreading side to side, producing new tumors far from the point of origin. Accumulating these mutations over the years permanently transform the function of the normal cells to become abnormal or Cancer cells. if you are diagnosed with brain cancer today, you did not get the cancer yesterday. Once the mutated gene is identified by MRI in the damaged organ. We design drugs to shut off the damaged genes.

In the laboratory of Professor Ross of London University, Chemists try to design drugs to shut off the mutated gene. By trial and error, from the hundreds of coloring agents, Ross selects a dye, which specifically stain the cells carrying the mutated gene. Using that dye, Ross attached Nitrogen Mustard (Bis-Chloroethyl Methyl Amine), a highly toxic substance used during the World Wars. Professor Ross observed that soldiers exposed to Nitrogen Mustard showed a sharp decrease in the lymphocyte count. Since patients who suffer from Leukemia (cancer of the blood) showed enormous increase in lymphocyte counts which are mostly premature lymphocyte, he thought that by using controlled amount of Nitrogen Mustard he would be able to control the formation of premature lymphocyte. Indeed, he was found to be correct. By attaching Nitrogen Mustard to amino acid Phenyl alanine, he synthesized Chlorambucil which is the most successful drugs used to treat Childhood Leukemia. Thirty years later, patients who were children at that time show no sign of Leukemia. His group synthesized large number of analogs of Nitrogen Mustard to treat a variety of Cancers. Using C-14 radiolabeled Nitrogen Mustards, Ross demonstrated that Nitrogen Mustard cross-link both strands of DNA shutting off the mutated genes. While Professor Ross designed drugs to cross-link both strands of DNA, as his student, I was to design drugs to attack a single strand of DNA.

In Professor Ross' Lab, I was trained to design drugs to attack mutated DNA both double stranded as well as single stranded DNA to shut off mutated genes. Professor Ross had spent all his life working on double stranded DNA published in a book called, "Biological Alkylating Agents" and also published a series of papers [7-13]. Using the same rationale, I worked with Professor Ross for almost ten year at the London University developing anti-cancer drugs. Instead of cross-linking DNA with Nitrogen Mustards, as his doctoral and postdoctoral students, I was assigned to use Aziridines to bind to a single strand of DNA shutting off the genes. There are two advantages of using analogs of Aziridine. First, it gives stability and second it gives selectively. Aziridines are stable in neutral and basic medium; it becomes activated in the acidic medium. Aziridines analogs attack only cancer cells as the cancer cells grow rapidly using Glucose as a source of energy. Glucose is broken down to Pyruvic Acid, which activates by opening the Aziridine ring to attack cancer cells. Aziridine rings open to generate a Carbonium ion. Where the Carbonium ion would attacks either the DNA nucleotide or Histone protein? If it attacked Histone Protein, it could turn the gene off temporarily and it can be reversed by adding 5-azacitidine. On the other hands if it attacked DNA, the binding is permanent and cannot be reversed. Aziridine shuts off gene permanently; the radio labeled study showed that Aziridine Carbonium ion attacks N-7 of Guanine nucleotide. I designed drugs to shut off abnormal genes either cross-ling both strands of DNA or binding to a single strand of DNA using Aziridine and Carbamate.

Method

During ten years period in the Professor Ross' Lab, I conducted several hundred experiments which resulted in 120 Aziridine analogs. All of them attack an experimental implanted solid animal tumor called the Walker Carcinoma 256 in Rats. Among all the tested analogs, the most effective drug was called CB 1954 (2-4, Dinitrophenyl Aziridine Benzamide). (See structure in Exhibit # 3). It was 70 times more toxic to the tumor cell [14-16]. The most effective drug ever made against the solid experimental tumor for which I was honored with the Royal Cancer Hospital's Institute Cancer Research post-doctoral award of the London University.

Why we must attack mutated DNA, because the Codons of mutated DNAs, code for wrong amino acids which produces wrong protein and which causes abnormal growth leading to cancers. The reason why we work on mouse model is that if you would compare the Genome of Man, Mouse and Monkey, they are all mammalians and their genomes are very similar. Once you succeed in attacking mouse tumor, it opens gate to attack Human tumors.

Genomic Medicine

Novel drugs designed based on the genetic make-up of a patient is called the Genome Medicine. My next challenge is to translate the animal work to human. I continued my work on the highly toxic Aziridine/Carbamate combination in America when I was offered the Fogarty International Fellowship Award to continue my work at the National Cancer Institute (NCI) of the National Institutes of Health (NIH). My supervisor, Professor Ross of London University was making useful novel drugs to cross link both strands of DNA, drugs such as Chlorambucil for treating Childhood leukemia, or Melphalan for treating Phalangeal Carcinoma, As I said above, my doctoral work at the London University involved attacking one strand of DNA using not only using Aziridine, but also using Carbamate.

My greatest challenge at NCI is to translate the animal work, which I did in London University to humans. One day, I came across a paper, which described that radio labeled Methylated Quinone cross the Blood Brain Barrier in mice. The X-ray photograph showed that the entire radioactivity was concentrated in the Mice's Brain. I immediately realized that a mutated gene called *methyl guanine methyl transferase* (*MGMT)* is responsible for causing Glioblastoma multiforme, the brain tumor in humans. Glioblastoma is a grade IV, CNS (central nervous system) tumor that grows and spreads very quickly. It is the most common primary brain cancer in adults. It can occur at any age, but is more common as we get older. The diagnosis of a glioblastoma includes a neurological exam, diagnostic MRI imaging, and a biopsy or surgery. It is a solid aggressive tumor like Walker Carcinoma in Rats. I decided to use Quinone moiety as a carrier for Aziridine rings to attack Glioblastomas. By introducing an additional Carbamate moiety, 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.

With the Quinone rings, which cross Blood Brain Barrier, I could introduce different combinations of Aziridine rings and Carbamate moieties and could create havoc for Glioblastomas. My major concern was how toxic this compound would be to the normal human brain cells. Fortunately, brain cells do not divide, only cancer cells divide. To grow, cancer cells use Glucose as a source of energy. Glucose is broken down to produce Pyruvic Acid. It is the acid, which activates the Aziridine and Carbamate moieties generating Carbonium ions attacking Glioblastomas.

Synthetic Medicine

As I said above, our Rational Drug Design using Aziridine/ Carbamate work began in the University of London, England, and completed in the Laboratory of the National Cancer Institute (NCI), of the National Institutes of Health (NIH), in Bethesda, Maryland, USA. Over a period ten years from UK to USA, we conducted over 500 experiments which resulted in 200 novel drugs. They were all tested against the 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). [17,18]. One of them is AZQ. Radiolabeled studies showed that AZQ has the ability to cross organ after organ, cross the Blood Brain Barrier, cross the nuclear membrane and attack the nuclear DNA shutting off the gene. Synthetic method of AZQ is described in detail in the US Patent 4,233,215. X-ray studies showed that the radioactivity is concentrated in the tumor region. Glioblastoma stop growing and start shrinking [19].

Our next challenge is to identify which genes on which chromosomes are attacked by AZQ to reduce the size of Glioblastoma. 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 three different kinds of brain cancers in humans. 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) is also implicated in some forms of Glioblastomas.

All known Glioblastomas causing genes are located on five different Chromosomes and collectively they carry a total of 9,579 genes. It appears impossible to design drugs to treat Glioblastomas since we don't know which nucleotide on which gene and on which Chromosome is responsible for causing the disease. With the completion of 1,000 Human Genome Project, it becomes easier. By simply comparing the patient's Chromosomes with the one thousand genomes, letter by letter, word by word and sentence by sentence, we could identify the difference variants with precision and accuracy, the exact variants or mutations responsible for causing Glioblastoma. It is daunting and time-consuming task. I developed a quicker method. Radiolabeled C-14 Aziridine will identify which genes on which Chromosomes is attacked by AZQ. The radiolabeled moiety will quickly identify which gene on which chromosome is attached.

A literature search shows that the International Scientific Community recognizes the significance of Dr. Khan's work. Using AZQ, they published more than 300 research papers in scientific literature. NIH considers his work is so valuable and innovative that he was honored with the "2004 NIH Scientific Achievement Award" one of the America's highest awards in Medicine.

He was also honored by the Government of India 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.

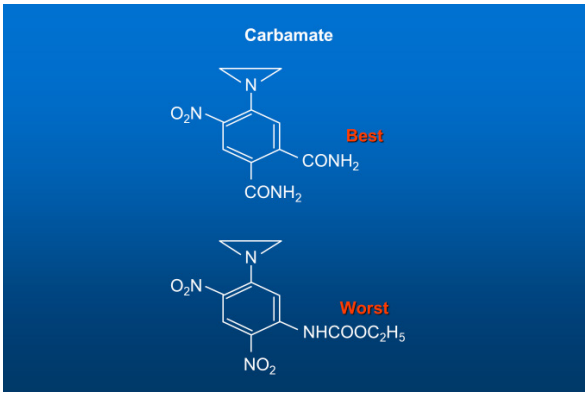
Exhibit # 2

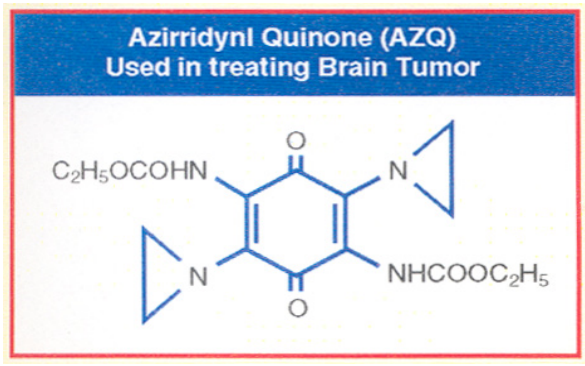
His Excellency, Dr. A.P.J. Abdul Kalam,
The President of India
Greeting
Dr. A. Hameed Khan

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

Single Strand DNA Binding Aziridine and Carbamate





U.S. Patent 4,146,622

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.

If we start getting MRI scans of the Glioblastoma of the patients every year, we could capture the progress of the disease at its earliest developmental stage. By comparing the patient's scans with Reference Sequence, we could identify the mutation and start treating the patients with AZQ as early as possible; we may be able to control the disease and prevent its progress to metastases.

What other Cancers should be explored next?

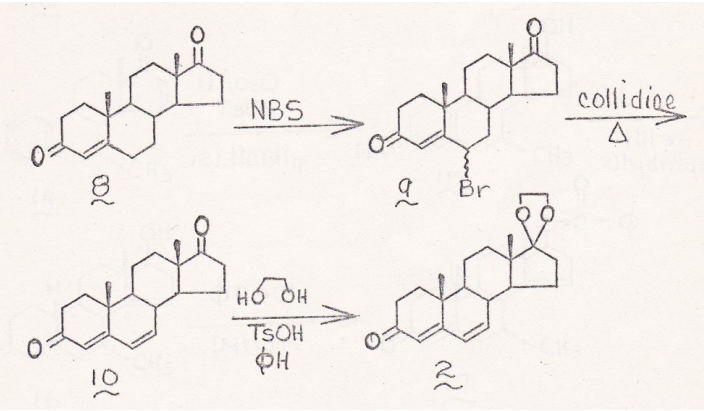
Of all cancers, the largest killer of women is the Breast and Ovarian Cancers. In spite of the use of highly advanced treatment methods such as Chemotherapy, Radiation therapy and Surgery, within three years of treatments, the tumor returns as metastatic cancer and kill the patient. There is no line of demarcation between normal and abnormal cells; these cells are intensely integrated. Surgery and radiotherapy cannot remove every abnormal cell. Within three years of surgery or radiotherapy, the few remaining mutated cells will grow rapidly and return as metastatic cancer cells attacking all organs killing the patients. Surgery or radiotherapy will not help, but chemotherapy could. Even if MRI identify the mutated BRCA-1 gene, it appears impossible to shut off that particular gene without affecting other genes. We could use the same rationale as was used for designing AZQ to treat Brain cancer.

Radiolabeled male hormone testosterone injected in female mice shows in X-ray, the concentration of radioactivity in the Breast and Ovary of the Mice. We could use Testosterone as a carrier for Aziridines and Carbamate for attacking the abnormal BRCA-1 genes and Ovarian tumor cells. Of the seventeen positions available in Testosterone, only three active positions are available and they are C-1, C-3 and C-17. The hydroxyl groups of all three positions could be chlorinated with Phosphorus penta-chloride in Phosphoric Acid. The trichloro-testosterone could be reacted with Aziridine and Carbamate in basic solution.

On the rational basis, I propose the following approach to develop novel drug to treat Breast Cancer. Although mutations on BRCA1 gene responsible for causing Breast Cancer located on Chromosome-17 has been identified years ago, so few drugs were designed on rational grounds. Now, we have sequenced Chromosome-17. We found that it is made of 92 million nucleotide bases pairs carrying 1,394 genes. By comparing with the Reference Sequence, we can easily identify which nucleotide on which gene of the Chromosome-17 is responsible for causing Breast Cancer. As I said above, Genomic medicine is a predictive medicine. By MRI (Magnetic Resonance Imaging which take three-dimensional images) and gene sequencing, we should be able to predict if the abnormal changes in the cellular DNA will lead Breast Cancer. Without this knowledge, it has been so difficult to design drugs on rational basis to treat Breast Cancer. By the time the Breast Cancer diagnosis is confirmed in a patient, the BRCA1 gene has accumulated more than three thousand mutations. Genotyping of the blood sample would also show the existence of many cells carrying mutated cells responsible for creating secondary deposits. It is also found in some cases when not detected earlier, by the time Breast Cancer is confirmed, metastatic cancer cells have already been spread from Liver Lung on their way to Brain.

As a Fogarty International Postdoctoral Fellow at the NCI, I was given the chance to work on any cancer, I pleased. Since all other organs including Breast and Liver could be removed and replaced by organ transplant except Brain, I thought that protecting Brain is utmost important to save life. For years, I work on the development of AZQ. Once the AZQ was developed to protect the Brain Cancer, I could focus on the Breast and Prostate Cancers. 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 radiations, 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 the three-dimensional MRI, we could show how many radio-labeled nucleotides were bound to which mutations. As I aid above, out of seventeen positions available for substitutions on Testosterone ring system, there are only three positions that is 1,3 and 17 are available for substitution on Testosterone ring system.



Carl Djerassi [20] 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 further increase 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 the number of Aziridine and Carbamate ions to get the maximum benefit by using Djerassi' method to activate Testosterone ring systems, we could develop novel approach to designing drugs to treat Breast and Prostate cancers which is based on the genetic make-up of a patient Genome to treat metastatic cancers.

Conclusion

After spending $3 billion on the Human Genome Project, if you would ask me what is the single most important discovery we made. Reading the entire book of our life itself is one of the greatest discoveries of our time. It will keep our scientists busy for another century trying to find out what piece of genome came from what species over three and a half billion of biological evolution. Of dozens of discoveries we made, one stands out. Sequencing the Human Genome helps us to explore the inner world of our body. MRI provides vivid images of each organ. If abnormal microscopic mutations are detected at its earliest, we could develop genomic medicine based on the genetic make-up of those organs. MRI will ensure parents that the unborn child is free from any genetic defect and would be an acceptable member of the human society. What if the fetus shows abnormal development? Should we start treating the child before it is born or after it is born. We have the technology to replace bad genes with good genes. While replacing the bad genes, could we introduce super genes to enhance the ability of the child such as high IQ, and athletic abilities knowing full well that these traits will be passed on to the future generations. Germ-line gene therapy is forbidden. Do we have rights to make genetic changes in children who will not even be born for another hundred year? New knowledge could create new problems, but knowledge is always superior to ignorance. We have a new problem, which provides a new solution.

Mitochondria are the powerhouses of our body. They provide energy to run our lives. Mitochondria carry smaller curricular DNA of about 16.5 K. bases pairs long located outside our genome. It is a DNA of a procaryote captured by our body millions of years ago. It lives in a symbiotic relationship with our body; it gets free food and housing and provides us with unlimited energy. Mutations in Mitochondria are responsible for causing horrendous genetic defects. Early detection by MRI could prevent mitochondrial disorder by nuclear transfer experiments; we have successfully conducted a three-parent conception in Monkeys in which fertilized egg is transferred in the egg of a closely related Monkey whose egg's nucleus has been removed. The good news is that the three-parent Monkey is free from genetic defects, but the bad news is that it will pass on its genome to future generations. The experiments have successfully been conducted in other animals, but not in humans for ethical reasons. Will the society accept the three-parent children free from mitochondrial diseases?

We need new ethical principles based on the modern science. We need to debate and discuss these issues and provide guidelines for the bench scientists. If MRI of all progressive diseases of all organs are made available to scientists designing drugs, it would help us develop treatment at its earliest. If this happens, the dawn of a new day will at lost long shine on the medical world.

Author

Dr. A. Hameed Khan was born in India, educated in England and received his doctorate degree in Organic 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. He moved to America when he was awarded 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) for which he was honored with the "2004 NIH Scientific Achievement Award" one of America's highest Award in Medicine. He was also honored with India's national Medal of Honor, a Gold Medal (Vaidya Ratna) by the Government of India. He is a Fellow of the American Institute of Chemistry and was elected to the American Science Advisory Board. He works at NIH.

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