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The Impact of Sequencing Human Genome on Epigenetic Diseases

Dr. A. Hameed Khan, Ph.D. (London)

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

***Corresponding Author:**

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

E-mail: Hameedkhan111@comcast.net

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Abstract

The purpose of this abstract is to identify Epigenetic diseases in Human Genome. After sequencing the human genome, we found that Methylation, Acetylation and Phosphorylation for regulating gene functions, are the common and widely used mechanism for epigenetic modifications; any abnormal mutations in epigenetic genome result in cancers, autoimmune disorders, neurological disorders such as Fragile X syndrome, Huntington Disease, Alzheimer, Parkinson diseases and schizophrenia. The essence of life is information and the information is located on four nucleotides, called Adenine (A), Thiamine (T), Guanine (G) and Cytosine (C). A string of these molecules is called the Deoxy Ribonucleic Acid (DNA). Since the Genetic and Epigenetic molecules are made of the same four heritable nucleotides, any mutations in genetic or epigenetic traits are stably heritable phenotype resulting from abnormal changes in a chromosome without alterations in the DNA sequence. Like Genetic, Epigenetics traits cover the modifications of not only DNA, but also DNA-binding proteins, and histones, which are important factors in making changes in chromatin structure without any change in the nucleotide sequence of a given DNA. Epigenetic mechanisms are involved in regulation of all biological processes in the human from conception to death. Like genetics, Epigenetic modifications often happen during an organism's lifetime; however, these changes can be transferred to the next generation if they occur in germ cells. After fertilization, the Genome sequencing of the embryo, will identify if the fetus is carrying any incorrect epigenetic marks resulting in birth defects. Epigenetic mechanisms also regulate development and adaptations. These alterations may result in various disorders including cancers. Since both genetic and epigenetic diseases are caused by the DNA mutations, the treatments of these diseases should also have the similar rationale such as gene therapy for diseases of a single nucleotide mutations or drug therapy for diseases of multiple gene mutations. By making AZQ (US Patent 4,233,215), I have demonstrated how to design drugs to shut off genes responsible for causing Glioblastoma, the brain cancer. It is the challenge for the next generation of scientists (my students) to develop drugs to treat all epigenetic diseases.

Keywords: Epigenetic disorder, Methylation, Acetylation, Phosphorylation, Glioblastoma. BBB, Aziridine, Carbamate, AZQ

I. A Note to my readers: The Impact of Sequencing Human Genomes are a series of lectures to be delivered to the scholars of the National Youth League Forum (NYLF) and the International Science Conferences. NYLF scholars are the very best and brightest students selected from all over the USA and the world brought to Washington by Envision, an outstanding organization that provides future leaders of the world. I am reproducing here part of the lecture which was delivered at the International Science Conference that was PCS 6the Annual Global Cancer Conference held on November 15-16, 2019, in Athens, Greece.

II. Special Notes: I am describing below the use of highly toxic lethal chemical weapons (Nitrogen Mustard) which was used during WWI and its more toxic analogs developed as more toxic weapons during WWII. I described the use of Nitrogen Mustard as anti-cancer agents in a semi-autographical way to accept the responsibility of its use. When we publish research papers, we share the glory with colleagues and use the pronoun "We" but only when we share the glory not the misery. In this article by adding the names of my coworkers, the animal handers, I will share only misery. The Safety Committee is interested to know who generated the highly lethal Chemical Waste, how much was it generated and how was it disposed. I accept the responsibility. The article below sounds semi-autobiographical, it is,

because I am alone responsible for making these compounds of Nitrogen Mustard, Aziridines and Carbamate. To get a five-gram sample for animal screening, I must start with 80 grams of initial chemicals for a four-step synthesis. To avoid generating too much toxic chemical waste, instead of using one experiment with 80 grams, I conducted 80 experiments with one gram sample, isolating one crystal of the final product at a time. The tiny amount of waste generated at each experiment was burned and buried at a safe place according to safety committee rules.

III. Ancient References that can be Googled on your cell phone are removed.

Historical Background:

On his farewell speech, my boss, the Director of the National Institutes of Health (NIH), Nobel Laurate, Dr. Harold Varmus, stated the following: At the Dawn of new millennium, we embarked on three great scientific revolutions in science. A Quantum Revolution, a Computer Revolution and a Genetic Revolution. These revolutions deal with matter, mind and life.

First, is a quantum revolution, we solve the mystery of matter. We discovered that the total matter in the Universe if made of atoms. We studied a single atom. We discovered that around centrally positive charge proton, revolves a negatively charged electron carrying a unit amount of energy called the quantum energy. It is the nucleus which is the heaviest and which carries the positively charged protons held together tightly by nuclear forces. If you break the nuclear bonds by bombarding with a stream of neutrons, as soon as the bond is broken, the protons fly apart hitting the neighboring protons setting a chain reaction releasing enormous amount of energy. With a ten-pound U-235, we release so much energy, it burns to ashes in minutes modern cities like Hiroshima and Nagasaki. If we control the chain-rection by placing a fine sheet of element Boron which absorbs protons, we can convert nuclear energy into electricity. In quantum revolution, the greatest lesson we learned is to convert matter into energy. Energy which runs the engine of modern society.

Second, the computer revolution. It is the revolution of mind. It brought the information age. Using two digits, zero and one, we wrote programs to convert the analog language of biology into the digital language of computer. We translated total information we generated since dawn of human civilization to the present day, into digital language of computer. Using computer, we develop information superhighway, we have the ability to retrieve, read, restore and return the information within a fraction of time. The greatest discovery we made in computer revolution is to capture space time. Using cell phone, we can contact anyone around the world in seven seconds.

Third is the genetic revolution, the greatest revolutions of them all. We solve the mystery of life. Now, we know with fairly certainty how life was evolved at some remote corner of the Earth about three and a half billion years ago. It crawled on the evolutionary path for about three billion years. When it became so advanced that, it could fly at will or stay still but it evolved on Earth from the ingredients already present on Earth. Three and a half billion years journey across time, it reached us. It helped us developed our language, our mind and our conscientious. We are the most intelligent creatures on Earth. We traced the beginning of life on Earth. A million-lightning strikes Earth each day. At some remote corner of the Earth, a cloud of gases containing Hydrogen, Oxygen, water. Methane, ammonia on a phosphate rock, a lightning struck creating an organic molecule called nucleotide, the first information molecule, the nucleotides were formed resulting in the RNA world.

RNA World:

Millions of nucleotides joined to form a self-replicating complex molecule called RNA (Ribonucleic Acid) the first information molecule for creating life. Our early Earth was filled with Nitrogen gas coming from millions of volcanos on hot Earth. Anaerobic life thrives on Nitrogen filled RNA World. Anaerobic life can store information like DNA and catalyzed reactions like proteins. According to Darwin, life evolved, and nature selected. Millions of nucleotides are created on the early Earth. When 120 to 90 thousand nucleotide base pairs joined together in an organism to form a new organelle called Chloroplast. It has a unique ability to conduct Photosynthesis that is, in the presence of sunlight, it can absorb atmospheric Carbon dioxide to convert to its food Carbohydrate and release Oxygen as a by-product. Oxygen gas is very toxic to the anaerobic life-forms. As more and more Oxygen is released, more and more anaerobic life-forms died. With the arrival of Oxygen by plants, RNA world ended, and more complex life-giving molecules were evolved such as DNA (Deoxy Ribonucleic Acid) a more stable molecule which store information, Proteins which carry out body function. Over eons, new molecules appeared such as Carbohydrates to provide energy, and Hormones to support life. These are all scientific facts. Now we know the answers to these questions where they all come from not from Heaven but were formed on Earth. The most stable among them is the information carrying molecule DNA.

DNA World:

The evolution of the present-day DNA world is due to the evolution of Chloroplast in plant kingdom. Chloroplasts are organelles present in plant cells and some eukaryotic organisms. Chloroplasts are the most important plastids which is a major double-membrane organelle found in the cells of plants and algae. Plastids are the site of manufacture and storage of important chemical compounds used by the cell. It is the structure in a green plant cell in which photosynthesis occurs. It is a primary site for forming essential amino acids Codons. The entire nucleotide sequence (the number and the order of the nucleotides) of Chloroplast Genomes has been determined. It is found to contain120-190 thousand nucleotide base pairs. While a typical plant cell might contain about 50 chloroplasts per cell, most land plant chloroplast genomes typically contain around 110-120 unique genes. Some algae have retained a large chloroplast genome with more than 200 genes, while the plastid genomes from non-photosynthetic organisms may retain only a few dozen genes.

During the last one hundred year, we discovered several sub revolutions. The first is the New World Order based on Crick Watson's structure of the double stranded DNA world [1]. According to the New World Order, life did not come to Earth from Heaven. All evidence indicate, Life is evolved on the surface of Earth from the ingredients already present on Earth. The second sub-revolution was the Gregor Mandel's work. When Mandel cross-bred red flower pea plant, with White flower pea plant. The next generation of flowers were all Red (Dominant allele) White flower disappeared (Recessive allele). When he cross bred muted first generation, two third red flower plants and one third of White flower plant returned. He called the red flower plant as Dominant allele and the White as Recessive allele. The White flower did not blend or mixed, the traits traveled in its entirety. What is true with plants is also

true with humans. Intermarriage among close relations., bring the defected member back in the third generation.

Next Darwinian revolution is extremely slow. To modify a species, it takes tens of thousands of generations to bring the changes in the species by environmental conditions. By using CRISPER-Cas9's method, we can edit genome that is by cutting, pasting and copying a specific gene, we can bring evolutionary changes in one generation. Next, Researchers have succeeded on a seemingly simply new concept of sequencing DNA by measuring current while threading DNA through Naturally occurring bacterial membrane protein which is called the Nanopore. The Nanopore sequencing method provides a means of detecting the sequencing of nucleotides in real time. They place a protein nano pour on a flat polymer membrane such that the only way DNA can travel across the membrane is through the tiny pores. When electric current is passed across the membrane, it measures the resistance change in current as the DNA snake through the pores at a speed approaching 500 nucleotide bases per second; next by using some clever informatic tool such as recurrent neutral network to convert the current measurement to nucleotide base identities; they succeeded in developing power to deduce genetic sequences in real time by translating the current fluctuation in the code of life.

Nanopore sequencing is a unique, scalable technology that enables direct, real-time analysis of long sequence of DNA or RNA fragments. It works by monitoring changes to an electrical current as nucleic acids are passed through a protein nanopore. The resulting signal is decoded to provide the specific DNA or RNA sequence faster, cheaper than any sequencer.

Next, we succeeded in conducting Fusion Reaction, by fusing Deuterium with Tritium, we generated 2 atones of Helium ions and an extra electron. The success of the reaction will provide unlimited source of energy needed for deep space travel.

Today, we have read that is mapped and sequenced the genomes of dozens of living creatures and identify not only the number of genes on a chromosome which occupy less than 2% of the chromosome, but also the total number of nucleotide bases and their order in which they are arranged in a species. The scripts of all living creatures are written in the same four nucleotide bases that is A-T and G-C. The traits we inherit from our parents are written in the same four nucleotide bases. The language of life shows that a kin relationship exists among all living creatures. If you sequence and compare the genomes of any two people, you find that our book of life is 99.9% the same and if you compare our genome with our closest relation, the Chimp, in the animal world, you find that our genome has 98.9% of the sequence of the genome the same as Chimp. Just 1.1% difference gives us intelligence and conscientiousness and makes us aware of our surroundings. Minutes difference between our genomes makes all the difference, we are free, and they are in the cages. If you line up the Sequence of human genome with of the genomes of many other species, you find that chromosome #20 in human is same as the chromosome #2 in mouse. The chromosome # 4 in human is aligned with chromosome # 5 of mice. If you aligned the sequence of human genome with fish, fly or worm genomes, you find a large section of human chromosome matches with the fish, fly or with the worm genome letter by letter. This is also true with all living creatures including plant's Chloroplast. A chloroplast is an organelle within the cells of plants and certain algae that is the site of photosynthesis, which is the process by which solar energy from the Sun is converted into chemical energy for growth of living creatures. The most important function of the chloroplast is to synthesize food by the process of photosynthesis which has the ability to absorbs light energy and converts it into chemical energy. Chloroplast has a structure called chlorophyll which functions by trapping the solar energy and is used for the synthesis of Carbohydrate food in all green plants.

Chloroplast is one of the three types of plastids. As I said above, the chloroplasts take part in the process of photosynthesis, and it is of great biological importance. Animal cells do not have chloroplasts, but they have Mitochondria. All green plant take part in the process of photosynthesis which converts Carbon dioxide into carbohydrates its food in the presence of sunlight energy and the byproduct of the process is Oxygen that all animals breathe. This process happens in chloroplasts. The distribution of chloroplasts is homogeneous in the cytoplasm of the cells and in certain cells chloroplasts become concentrated around the nucleus or just beneath the plasma membrane. A typical plant cell might contain about 50 chloroplasts per cell. The extinction of anaerobic life forms in the presence of Oxygen paved the way for a burst of new life, called the Cambrian explosion.

Over eons, planet Earth began to warm and in the presence of Oxygen atmosphere, the appearance of first single cell Pre-Cambrian creatures that attack each other forming a multicellular creature. These earliest forms of life resembled Cyanobacteria. They were photosynthetic blue-green algae that thrived in the extremely hot, carbon dioxide-rich atmosphere. For millions of years, the job of blue green algae was to perform photosynthesis that is to absorb Carbon dioxide and release Oxygen which is essential for keeping the creatures of DNA world alive.

Essential components of life are RNA, DNA, Proteins, Carbohydrates, Lipids, and Hormones. We always wonder how these non-living chemicals could get together to create living creatures. When did Chemistry became Biology? When did life evolve? Where was it evolved? And how life was evolved? Evolution of Life on Earth is not a miracle. Life could have been evolved on Earth's surface such as on the oldest rocks found in Australia or it could have been evolved at the bottom of the Ocean floor where Black Smokers are formed with Lava emerging from under sea volcanoes reacted with surrounding Hydrogen Sulfide gas which provides energy for life forms such as tubeworms and crabs that thrive on the Ocean floor. Life also could have been evolved underground. Soil sample brought by miners from the gold mines in South Africa two miles deep underground contained micro worms. Such life form could be cultivated on a Petri dish containing Agar mixed with nutrients. Early life could have been unicellular. Could life have been brought on Earth by meteorites. Early Earth has no Water. Billions of Comets brought Water on Earth. Would it be possible that some of those Icy comets contained life giving essential components? Life could also have been evolved on the surface of Earth. The polymerization of Formaldehyde in the atmosphere could produce Carbohydrates another essential component of life. The presence of Acetonitrile, Carbon dioxide, Water in the presence of Ultraviolet light could produce the nucleotides such as Adenine (A), Thiamine (T), Guanine (G) and Cytosine (C) forming a binary code leading to RNA which start replicating itself creating the first living anaerobic creature. Since no human was present to witness the formation and evolution of first life on Earth, we rely on its presence from the early fossils found in the layers of ancient rocks.

Once a single replicating living cell appears on Earth, complexity develops. In other words, all complex life forms

are evolved from simpler life forms. Fossils are the remains of the pre-historic life forms. To become fossilized, the species must have developed hard parts such as bone or shell and must be trapped in mud which slowly become hard rock. Soft tissue creatures do not fossilize; their tissues decomposed. The first life form appeared on Earth about a billion year after the Earth was formed about four and a half billion years ago. Over billions of years of evolutionary process give enough time for the chemicals to react together to create Life.

Technology developed faster during the last hundred years. Sequencing provides the complete text of our genome. By using the methods of genetic engineering, we know where to cut, paste and copy a gene to create new synthetic life. Synthetic life will help us move genes from species to species from plants, to man, to mouse to monkey and to microbes to produce new food, new fuel and new medicines to treat every disease known to mankind.

Genetic Engineering:

On a long string of DNA, out of four-letter text, three nucleotide code for an amino acid called codon. All four genetic letters give 64 codons which code for all 20 amino acids. A gene is a unit of inheritance and it is identified on the long string of DNA by the start codon AUG which codes for amino acid Methionine. After several dozen codons to code for a gene, the stop codon appears to stop the extension of DNA. There are three stop codons and they are UAG UGG and UGA. Once the genes are identified on a long string of DNA, our next challenge is to cut, paste and copy a single gene. The product of a single gene is a protein which could be used to treat a disease such as Insulin to treat diabetes.

The tools of genetic engineering were developed by Bacteria. Over eons, bacteria have learned to fight back the Phages by producing an enzyme called the Restriction enzymes which acts as molecular scissors for cutting the DNA into pieces. A couple of hundreds of Molecular scissors (called Restriction enzymes) were discovered during war between Bacteria and Viruses. A restriction enzyme is a protein isolated from the bacteria that kill viruses by cleaving DNA sequences at sequence-specific sites, producing DNA fragments with a known sequence at each end. Several hundred restriction enzymes have been isolated which cut DNA at specific sites. Restriction enzymes, also called restriction endonucleases, recognize a specific sequence of nucleotides in double stranded DNA and cut the DNA at a specific location. Individual genes from these fragments are isolated on Gel Electrophoresis They are indispensable to the isolation of genes and the construction of cloned DNA molecules. Restriction enzymes can be used to splice and insert segments of DNA into other segments of DNA from any other species, thereby providing a means to modify DNA and constructing new forms.

The use of restriction enzymes is critical to certain laboratory methods, to cut paste and separate a piece of DNA. Naked genes are unstable in biological fluid and are destroyed by enzymes. To protect genes, we make recombinants with Bacteria or Plasmid called Vector. In recombinant method, we generate two DNA fragments with matching ends in Plasmid or Bacteria. We can join them by the enzyme DNA ligase which seal the gap between the molecules forming a single transgenic plasmid for each gene. We use this method to prepare a Restriction site map to identify all genes on a chromosome. To make enormous copies of a gene. Restriction enzymes and DNA ligase are often used to insert new genes and other piece of DNA to make large number of copies into transgenic plasmids during DNA cloning. Transgenic Plasmids for each gene are prepared to store as a gene library for future use. Once harvested in Yeast or Bacteria, Transgenic plasmids are broken by Restriction enzyme to release pure proteins.

Once the role of restriction enzymes was established, the two times Nobel Laureate Fred Sanger, put together a non-replicating virus, accurately that read the number and the order of the genetic letters (sequencing) called Bacteriophage Phi X 174; it has 5,386 (Kilo-base) genetic letters to be précised in this creature. Scientists have successfully put those letters together other life forms in that exact order and injected to a bacterium. The unfortunate bacteria read its code as its own and inserted in its genome. Where Phage integrates its DNA into the host cell DNA and multiplies into thousands of its own copies and kills the bacteria. The phi X 174 bacteriophage is a single-stranded DNA virus that infects Escherichia coli, and the first DNA-based genome to be sequenced. Creation of first life form in the test-tube started a New World Order. Creation of life on Earth is no longer a magical, mystical process sent on to Earth from Heaven in seven days is over. Instead, according to the new world order, the creation of life on Earth is extremely slow evolutionary process taking billions of years. Geological records confirm this fact. The creation of life in the Lab opens the gates to create all kinds of useful life forms to produce new food to feed over eight billion people on Earth and provide new fuel to run the engine of modern society and new medicine to treat every disease known to mankind.

Using the restriction enzymes, scientists around the world, started isolating genes from various species patenting and claiming as their own. The US government rejected their claim. Instead, the US Government decided to provide funds to decipher the entire book of life of a human being called the Human Genome and release all the genes free of charge to anyone who wants it. Sequencing Human Genome, reading the number and the order in which these nucleotides are arranged is called sequencing. Sequencing will answer the most fundamental questions, we have asked ourselves since the dawn of human civilization. Questions like, what does it means to be human? What is the nature of our memory and our conscientiousness? Our development from a single cell to a complete human being? The Biochemical nature of our senses, the process of our aging? Scientific biases of similarity and dissimilarity. Dissimilarity that all living creatures from a tiny blade of grass to mighty elephant, including man mouse and monkey are all made of the similar building blocks the nucleotides and yet we are so diverse that no two individuals are alike even identical twin are no exactly identical, they grow up to become two separate individuals.

In 1990, US Congress authorized three billion dollars to our Institute (NIH) to decipher the entire human Genome 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 non-coding nucleotides forming 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 arranged 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.

A single cell is so small that we cannot even see with our naked eyes. We must use a powerful microscope to enlarge its internal structure. Under an electron microscope, we can enlarge that one cell up to nearly a million times of its original size. Under the electron microscope, a single cell looks as big as our house. There is a good metaphor with our house. For example, our house has a kitchen, the cell has a nucleus. Imagine for a moment, that our kitchen has 23 volumes of cookbooks which contain 24,000 recipes to make different dishes for our breakfast, lunch, and dinner. The nucleus has 23 pairs of chromosomes which contain 24,000 genes which carry instructions to make proteins. Proteins interact to make cells; cells interact to make tissues; tissues interact to make an organ and several organs interact to make a man, a mouse, or a monkey. To confirm the composition of nucleotides, we must sequence the entire human genome. As you know, every cell of our body carries sixteen thousand good genes, six thousand mutated (bad) genes responsible for six thousand diseases and two thousand Pseudo-genes that have lost their functions.

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

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 millions of new combination of proteins. The cells use new combination to generate new proteins as needed). It is estimated that less than 19,000 genes code for protein. Because of the alternative splicing, each gene codes for more than one protein. 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.,3., 4., 5., 6.,]

Our next step is to isolate proteins from the good genes and design drugs to shut off bad genes. We can isolate and manipulate a single gene from human genome. We can insert a single gene in the fertilized egg of an experimental animal in such a way that the new gene is turned on in the host cell producing a new protein. Using the restriction enzyme, (like EcoR1 which acts like molecular scissors), we cut down the chromosomes to pieces at specific sites. We separate and isolate a gene by gel electrophoresis. We prepare a restriction site map. Each gene is confirmed by comparing with the Reference Sequence. A Molecular Vehicle, Vector (such as disabled Viruses, Bacteria, or Plasmids), is created that will carry the gene into the nucleus of the cell where it permanently integrates into the genome of the host cell creating a trans-gene. As the cell begins to grow and divide, it makes copies of the trans-gene. For example, Insulin is isolated from a gene located on Chromosome-11 in Pancreas. It was harvested in large scale in either bacteria or Yeast. It is now used to treat 300 million diabetics around the world. Similar method could be used to make proteins from all 16,000 good genes of our genome.

Not all genes act simultaneously to make us function normally. Current studies show that a minimum of 2,000 genes are enough to keep human function normally; the remaining genes are backup support system, and they are used when needed. The remaining 2,000 genes are called the pseudogenes. For example, millions of years ago, humans and dogs shared some of the same ancestral genes; we both carry the same olfactory genes needed to search for food in dogs. Since humans do not use these genes to smell for searching food, these genes are broken and lost their functions in humans, but we still carry them. We call them Pseudogenes. Recently, some Japanese scientists have activated the pseudogenes, this work may create ethical problem in future as more and more pseudogenes are activated. Nature has good reasons to shut off those pseudogenes. Our Genome provides the genetic road map of all our genes, past, present and future. For example, it can tell us how many good or bad genes we inherit from our parents and how many of those gene we are going to pass on to our children. If a family has too many bad genes, and have a family history of serious illnesses, they can break off the flow of information either by stop having children or stop donating mutated eggs and sperms.

Reference sequence:

We can scan the whole genome (Reference Sequence) for its response to a given situation. When we look at a normal cell and compare with an abnormal cell, we see the differences or when we compare their gene expression looking for a specific protein, from a specific gene and for a specific nucleotide sequence, we can identify a specific mutation responsible for the disease. In pre-genomic era, before sequencing human genome, when a patient visits a physician for some unknown ailment, the Physician would order several tests and would say to his patient, I do not know what is wrong with you,

but I will see if any of these tests show if my guess is right and if he is wrong, he will recommend few more tests to see if he could identify the illness. The guesswork and the trial-and-error days are over. **Now, after sequencing the human genome, the physician would say to his patient, I do not know what is wrong with you, but I know where to find it. It is written in your Genome.** He would order the sequence of patient's genome. It would be easy for a Physician to scan the patient entire genome and compare against the Reference Sequence to identify the mutations responsible for causing the disease. He will refer the patient to a biotechnology Lab. The Lab Technician will take a small blood sample from the patient, separate his WBC, extract DNA, sequence his Genome and compare with the Reference Sequence letter by letter, word by word by word and sentence by sentence and send the result to the Physician who can easily identify the mutations responsible for causing the disease. The result will provide the best diagnostic method to identify a disease.

Our Genome is not just a diagnostic road map of our genes, it also tells us to clone the good genes and shut off the bad genes. Using the good genes, it also tells us to make its large-scale protein for worldwide use such as Insulin and Human growth hormone. On the other hand, identifying the bad genes and tells us to design novel drugs to shut off bad genes responsible for causing serious diseases. We have already demonstrated that using the genetic engineering techniques, we can cut, paste, copy, and sequence a good gene for making on industrial scale such as the production Insulin to treat 300 million diabetics around the world.

Genome sequencing of bad genes start a new era of Genomic Medicine which is based on the development of new drugs for treating a disease based on the genetic make-up of the individuals. The next step would be to design drugs to shut off the mutated genes. Gene Therapy will work if the disease is caused by a single gene mutation. Drug Therapy will work if multiple genes are responsible for causing diseases such as Cancers, Cardiovascular diseases, and Alzheimer.

The advantages of Sequencing Human Genome:

The knowledge gained by sequencing human genome has summarized the past 150 years of genetic science. We have taken away the power from Mother Nature to alter billions of years of our evolutionary past. We now have all the tools we need to alter the genetic make-up of our species. Genetic Revolution has taught us that Darwinian evolution can be hastened by the rules of genetic engineering. By using the genetic tool kits, we can cut, past, copy and sequence a gene in days not in eons. The development of new tools like CRISPER-Cas 9 for cutting, removing and replacing a bad gene with a good gene making it possible to edit the genes of all species including our own with far greater precision, accuracy, speed, flexibility, and affordability than ever before. Now, we control our own destiny. We ignore the scientific facts at our own peril.

One of the advantages of sequencing the personal genome is that after seeing our own sequence most of us will conceive our offspring in the Lab rather than in our bed. What they see in their personal genome is the three and a half billion years of random mutations whose ancestors have continuously outcompeted their competitor in a never-ending cage match of survival. From this point onward, no one will take an unnecessary risk. Our offspring will not carry random mutations. It will be self-designed. From this point onward, our selection will not be natural. It will be self-directed. The current version of our Homo Sapiens species will never be evolutionary endpoint, but always be a stop along the way in our continuous evolutionary journey. During the last few hundred years, we moved from Agricultural Age to Industrial Age and then from Atomic Age to the present Information Age. Now we are entering the Space Age trying to find out how to survive on exo-planets. More than five thousand exo-planets have been discovered so far.

The best advice for those couples who have a family history of long-term illnesses is to compare their personal sequence with the Reference Sequence. In the entire human genome, we find five thousand mutations responsible for causing five thousand diseases including mitochondrial diseases.. Each of us carry a single copy of at least five to six deleterious mutations; we are carrier, but if we marry someone who is also carrying the other copy, we are most likely to have a sick child. In the lab, before conception, we could sequence and discard a defected embryo to prevent the high cost of raising a sick child. The defected embryo can always be replaced by an embryo free from all mutations.

Some parents may consider the possibility of not just selecting the best embryo for in vitro fertilization but also to introduce superior traits to genetically altering the future of their children Although in vitro fertilization is encouraged to prevent the introduction of mutated genes in the gene pool, but introduction of gene enhancing traits in the germ-lines are not permitted at this time because these traits are passed on to future generations. The following studies are forbidden: For example, a combination of genes which impart long life, high athletic or singing ability, or to make them smarter and superior to the other children, or to the introduction of new genes which make them resistant to many infectious diseases, or to introduce genetic traits associated with genius, or animal like extra-sensory perception, or to synthesize new traits, not yet known in humans, but made from the same nucleotide sequence which give rise to great diversity of life,

Prolonging human life: (Such studies are not funded at this time). We need to sequence the Genomes of Centenarian who live beyond hundred years. By comparing their genomes with the Reference Sequence, we should be able to identify the rare allele which prolong their lifespan. Once identified the allele, we need to conduct genetic engineering that is to cut, paste, copy, and splice the allele into the Genome of volunteers to study its function.

The Human Genome Project showed that our Aging is a combustion process. The tail end of each chromosome carries a set of a six-letter code called Telomer. Aging is related to the loss of Telomeres, the six-letter code (TTAGGG) that shorten the length of our DNA in our chromosomes also shorten our lifespan. During replication, each Chromosome loses about 30 Telomeres each year. If we slow down the loss of Telomeres by using the enzyme Telomerase Reverse Transcriptase (TRT), we could slow down the aging process. We have already demonstrated in the worm C. Elegance that by using TRT gene, we have increased its lifespan by several folds. Now, we could translate this work first in mice then in human embryo; we could try by making a Vector, a virus, carrying TRT gene when infected the embryo and harvested to eight-cell and sequence to confirm the presence of the trans gene. The TRT gene would have been inserted in the entire genome of every cell of the growing embryo. By sequencing a single cell to confirm that the TRT transgene is spliced, we could implant TRT gene carrying embryo in the mice womb. If this transgenic experiment in mice is reproducible and verifiable, we could try in human embryo. Suppose this experiment conducted in humans is successful

and suppose the sequence show that at each replication only 15 Telomeres are lost instead of 30 Telomeres. Since the longevity treatment with the TRT transgenic virus is safe, inexpensive and would be easily available to human. Should we provide the treatment to every man, woman, and child on the face of the Earth or make it available to long distance space travelers only?

To control early symptoms of old age diseases, frequent genome sequencing will help us identify a single gene mutation that will begin to grasp more complex genetic patterns that could lead to polygenic or multigenic conditions such as Coronary Heart diseases, Cancers, and Alzheimer. Early detection will help us control their expansion. Some genes are activated at the later part of our life causing serious illnesses. If there is a family history of such diseases, frequent sequencing becomes more important for early detection.

With the development of the genetic toolkit, we can perform genetic engineering. We can separate good and bad genes. We can cut a good gene (using Restriction Enzyme such as EcoR1), paste a gene (using enzyme DNA ligase) and copy a gene in plasmids. Using recombinant technology one can prepare a trans-gene Vector either in plasmids or in bacteria and harvest them in Yeast or bacteria to make large quantity of protein such as Insulin to treat more than 300 million diabetics around the world. We can also move the gene from species to species or design drugs to shut of bad genes to treat diseases such as cancers.

On April 3, 2003, several groups simultaneously sequenced the entire Human Genome and confirmed that less than two percent of the Genome codes for proteins the rest is the non-coding regions which contains switches to turn the genes on or off, pieces of DNA which act as promoters and enhancers of the genes. Using restriction enzymes, we can cut, paste, and copy genetic letters in the non-coding region which could serve as markers, but a slight change in the coding region of the genome called mutations could make a normal cell abnormal or cancerous.

After Sequencing the Human Genome, our search for unknown diseases has come to a closure:

There are two most powerful implications of the human Genome Sequencing. One of them is that we have come to closure. What it means is that we have the catalog of all genes in the Human Genome, we can search the entire genome and locate the desired gene. we will not wonder in the wilderness anymore. Everything there is to know about human health and traits are written on these genes in nucleotide sequences. Our Genomes provides the catalog of all genes.

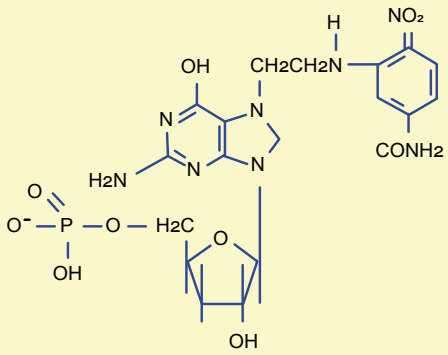
The second implication is that we can scan the entire genome against the suspect region of the genome to identify the mutation responsible for causing the diseases. Using the recently completed 1000-genome project, we can scan the suspect region a thousand time to identify the disease-causing nucleotide with precision and accuracy. Once the nucleotide is identified, it will point to the codon which codes for the wrong amino acid. The mutated codon will point to the gene which codes for wrong protein responsible for causing the diseases. The next step is to shut off that gene either by gene therapy, CRISPER or drug therapy.

Different Binding sites for Genetic and Epigenetic Diseases:

Since the genetic and epigenetic diseases are caused by mutations of the same four nucleotides, A-T and G-C, we could use the same rational to design drugs to shut off genetic as well as epigenetic diseases. The most powerful weapon developed during the World Wars was Nitrogen Mustards which attacked DNA nucleotides shutting off genes. Beside Nitrogen Mustards, other DNA attacking agents are Epoxide, Aziridines and Carbamates which generate powerful Carbonium ions which attack nucleotides. Methylating and Ethylating agents are the primary attacking agents to shut off a gene. In Epigenetic, methylation is the primary agent to shut off a gene or it can be de-methylated and regulate gene expression. Methylation gives high stability and serves as a special epigenetic memory of specific cells throughout all periods in the cell cycle. It may also regulate the expression and the activity of histone codes. Acceleration of DNA methylation at CpG sites (cytosine and guanine separated by only one phosphate group; phosphate links any two nucleosides together in DNA) .is mediated by DNA methyltransferase enzymes such as DNMT1, DNMT3a, and DNMT3b. Inside the cells, S-adenosyl methionine act, as an important methyl group donor. In this sense folic acid and B12 play the determinant roles in re-methylation or the attraction of de-methylated form of S-adenosyl methionine through passive and active mechanisms. **The important attacking site in epigenetic is Lysine residue.**

On the other hand, in Genetic drugs, the attacking agent to shut off a gene is Ethyl Carbonium ions such as Nitrogen Mustards, Aziridines and Epoxides. **The important attacking site is N-7 Guanine.** While Methyl group in Epigenetic is reversible, the Ethyl group in Genetic drugs are irreversible.

Conjugated DNA Disrupty Protein Synthesis Pathway of Cancedr Cell



Here I cite my own work [15., 16] for binding genetic drugs to DNA. During the radio-labeled studies of 2,4-dinitrobenxamide (CB 1954), the most active compound against the experimental animal tumor, Walker Carcinoma 256 in Rat, C-14 radio labeled studies showed that radio-active Aziridine binds to N-7 Guanine of DNA shutting off the gene. (see above structure). N-7 binding to DNA is irreversible.

Gene Therapy:

The first step is to cut the human genome with specific enzymes (prepare a Restriction Site Map) at the specific sites using restriction enzymes (molecular scissors such as EcoR1) first accomplished by El Salvador Luria, Max Delbruck, and Hamilton Smith. The fragment of human DNA (a single gene) if not protected will be destroyed by antibody. A naked gene is a piece of DNA (which has a start codon AUG and after a few thousand nucleotide (codons) end at one of the three stop codons UAG, UGA or UGG if not protected by recombinant

technology (making a hybrid) that is by recombining with the DNA of Virus, or Plasmids, or Chloroplasts (for plants) which serves as Vectors. If not protected it will be destroyed by enzymes. One can store the fragments or genes in the Vectors once the human DNA fragment is stabilized in Vectors by recombinant technology; we can not only purify this fragment (genes), but also, we can make millions of copies (clone) of this fragment of DNA by transferring into the host cells such as Bacteria, mammalian cells or Yeast cell which autonomously replicates to produce library of genes. Each Library contains millions of copies of identical genes that produce the same protein. Before the genetic revolution, Insulin is extracted from pancreas of the slaughtered animals which is used to treat old diseases such as diabetes; a tiny fragment of impurity could set anaphylactic shock and kill the patients. Now, large scale highly pure human Insulin produced by Genetic Engineering firm named Genentech is used to treat 300 million diabetic patients worldwide without the loss of a single life. Other products of Genomic Medicine such as Growth hormones and hormone proteins to treat Hemophilia by factor VIII protein are being developed as genomic medicines by recombinant technology. Attempts are being made to design drugs to attack cancer cells on all three levels that is DNA, RNA and Protein. Herceptin, a novel class of drug, has been successful in attacking protein. Craig Milo has designed double stranded RNA to shut off gene and prevents its translation into protein.

"CRISPR" Technology:"

CRISPER Stands for, "Clustered Regularly Interspaced Short Palindromic Repeats."

We recently develop a new technology to edit genome. It is called CRISPER-Cas9. It allows us to make changes in the DNA and cells, to cure genetic diseases. This technology is based upon basic research project aim at discovering how bacteria fight viral infection. When viruses infect a cell, they inject their DNA and, in a bacterium, the CRISPER system allows that DNA and inserted a little bit into the chromosome DNA of the bacterium. These integrated bits of viral DNA get inserted at a site called CRISPER. The viral bits of viral DNA are passed on to their progeny. Cell is protected for many generations. This allows the cells to keep a record of infection. Once a bit of DNA is inserted into the bacterial chromosome, the cells make a little copy of a molecule called RNA. It is an exact replicate of the viral DNA. RNA is a chemical cousin of DNA. It allows interaction with DNA molecule that has a matching sequence. Those little bits of RNA, from CRISPER locus associated bind to protein called Cas9 and forms a complex which functions like a sentinel in the cell. It searches through all the DNA in the cell to find sites that matches sequences in the bound RNA and those sites are found; it allows the Cas9 clever to cut up the viral DNA. Make a very precise break. We can think of Cas9 RNA sentinel complex like of a pair molecular scissor that can cut DNA make a double stranded break in the DNA helix. This complex is programable. It can be programmed to recognize a particular piece of DNA sequences and make a break in DNA.at that site. This technique could be used in genome engineering to allow cell to make a very precise change in DNA at the site where this break is introduced. It has the ability to detect broken DNA and repair it. The reason why we envision using CRISPER system to genome engineering is because cell has the ability to detect broken DNA and repair it. So, when the plant or animal cell detects a double stranded break in its DNA, it can fix that break either by passing together the ends of broken DNA or a little change in the or it can repair the break by integrating a new piece of DNA at the site of the cut. If we have to introduce a double stranded break into DNA at precise places, we can trigger those cells to repair those breaks either by the destruction or by incorporation of new genetic information. If we were able to program CRISPER technology to make a break in DNA at the position at or near a mutation causing Cystic fibrosis for example, we can trigger cell to repair that mutation

In summary, when a virus infects a bacterium, it has a few minutes to respond or it is destroyed. Most bacteria have adapted a new system called CRISPER that allows them to detect viral DNA and destroyed it. Part of the CRISPER system is a protein called Cas9 that allows us to seek out and cut the DNA and eventually degrade viral DNA in a specific way. In a search to understand the activity of the protein Cas9 that we realized that we could harness its function as genetic engineering technology a way for a scientist to read or insert specific bits of DNA into a cell with incredible precision. CRISPPER technology has already been used to changed DNA in the cells of mice. Chinese scientists chose recently used CRISPER technology to make change in the genes in human embryo. (forbidden).

To edit a genome, first, you have to break the double stranded DNA to pieces using restrictions enzymes like CRISPR-Cas9. Once the genome is broken, in a living organism, cellular repair processes set in to repair DNA. During the repair mechanism, we can make changes in the DNA structure by inserting, removing, adding, replacing, exchanging or editing DNA. It is a two-part system, a Cas-9 protein and a guided mRNA carrying complimentary sequence, which acts as guided missile to target the mutated section of DNA. When the target DNA is found, Cas9, which serves as a molecular scissors, one of the enzymes produced by the CRISPER system, binds to the DNA and Cas9 cuts the mutated DNA, shutting off the targeted gene. Cas-9 removes DNA, and guided mRNA searches until it finds the exact complimentary sequence. And the combination of the two, called CRISPR/Cas9. It works by a DNA sequence of a specific genetic location and deleting or inserting a new DNA sequence which can change the genome either by a single base pair of DNAs, or large pieces of chromosomes, or entire regulation of gene expression. The guided mRNA is only twenty letters long which targets the system and is easy to design. It is inexpensive if everything in the system stays the same. This procedure is remarkably easy and powerful to use. So, the guided mRNA and the Cas-9 protein together move along the genome until they find the mutation spot, where the guided mRNA matches, then it cuts and inserts the new piece of DNA between the double helix which separates the strands apart that trigger the Cas-9 protein to cut the double strands. To repair, it has two major repair pathways, first it puts the two pieces of DNA back together. In second repair pathway, it takes a homologues piece of DNA from any species and put it together.

In a diploid organism like us. We got two sets of DNAs in our chromosome; one copy from Mom and the other from Dad, if one copy is damaged, we can use a piece of DNA from the other chromosome to repair it. The repair is made and the genome is safe again. Similarly, we can hijack this process and can insert a new piece of DNA, a piece that has homologues at both ends and different in the middle. Now you can put whatever the sequence we want in the middle. We can change a sequence; we can either put or take a letter out of genome. You can insert different DNA sequence which acts like a Trojan Horse. We can put wherever sequence we want.in the genome.
Some guided RNA works well other do not. It is easier to do in a Petri dish on a single organism. For example, in leukemias patient, we can take the blood sample out, remove the mutated sequence and replace it with the corrected gene. The corrected

genome is harvest and put it back in the patient. To do safer on the entire human genome, you have to use Virus. You let the modified virus infect the cells and put it back in humans. It may work, but you have created a new problem You have insert a foreign piece of DNA in human, what is the long-term effect by introducing a new piece of DNA in your genome.

Drug Therapy:

Gene Therapy cannot be applied to treat diseases with multiple genetic defects such as cancers or heart diseases. Drug Therapy could be used to develop novel treatments. Since the genetic and epigenetic diseases are based on the same four nucleotides, A-T and G-C, we could use the same rational to design drugs to shut of genetic as well as epigenetic diseases. For example. the gene that codes for Insulin protein was identified on Chromosome-11 when we sequenced the genome of Pancreas. Chromosome-11 consists of 144 million nucleotide bases and carry 1,692 genes. One of those genes codes for Insulin which controls sugar level in diabetics. Today, more than three hundred million diabetics around the world use Insulin to control their sugar level. Other beneficial genomic medicines include Human Growth Hormones, Blood Factor VIII etc. We have a choice either to treat these diseases by genomic medicine or to identify and eliminate all seven thousand genetic diseases in human Genomes before conception. Couples with the family history of genetic diseases, should be asked to have their genome sequenced or their Egg (X-chromosome) and the Sperm (Y-chromosome) sequenced before conception to discard severely damaged egg and sperm before conception and to select healthy egg and sperm for in vivo fertilization. Compared to Egg, Sperm is very small. The X-chromosome in female is made of 164 million nucleotide bases and carry 1,144 genes while Y-chromosome in men is made of 59 million nucleotide bases and carry 255 genes. Using Nanopore sequencer, we should be able to sequence the X and Y chromosomes cheaper, faster with precision and accuracy. Comparing with the Reference Sequence data, we should be able to identify the mutations responsible for causing the genetic and epigenetic defects such as sickle cell anemia, muscular dystrophy, Hemophilia, Cystic Fibrosis, schizophrenia, bipolar disorder, Tay-sac etc. Once identify the mutated genes. If the mutation is caused by a single base mutation, we can treat them with either gene therapy or CRISPER-Cas9 therapy that is replacing the bad gene, but if the disease is caused by multiple genetic defects, gene therapy and CRISPER-Cas9 therapy will not work, drug therapy will that is to design new drugs to shut off the defected genes responsible for causing the disease.

The mechanism of action of p53 gene and the development of its treatment:

The gene p53 works in aggregate of four subunits and more identical copies of smaller subunits. All four subunits must work together and must act correctly in order of p53 to properly control cell manipulation. This solves the central riddle of cancer. This explains why it takes years to develop cancer after exposure to mutagens such as radiations, carcinomic material such as Asbestos, chemical/environmental pollution or viral infection or genetic inheritance. The reason it takes so long is that a series of multiple mutations must occurs before the growth mechanism of the cell is finally disrupted. The p53 gene like the Rb gene, is a tumor suppressor gene, i.e., its activity stops the formation of tumors.

To develop treatment for p53 mutated gene and to restore the function of p53 protein, we must sequence each of the four sub units and compare with the Reference Sequence to identify the damage to DNA. Even a single mutation in a single subunit could disrupts the production the entire p53 repressive protein. Mutation in each sub unit must be considered as responsible for damaging the function of all four sub units and damage to the entire p53 protein. Once the site of mutation is identified, you can follow the same rationale as I have described below in making AZQ.

If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early adulthood. Cell cycle arrest and apoptosis are the most prominent outcomes of p53 activation. Many studies showed that p53 cell-cycle and apoptosis functions are important for preventing tumor development. p53 also regulates many cellular processes including metabolism, antioxidant response, and DNA repair. Activated p53 promotes cell cycle arrest to allow DNA repair and/or apoptosis to prevent the propagation of cells with serious DNA damage through the transactivation of its target genes implicated in the induction of cell cycle arrest and/or apoptosis. Upon activation, p53 induces the expression of a variety of gene products, which cause either a prolonged cell- cycle arrest in G1, thereby preventing proliferation of damaged cells, or apoptosis, thereby removing damaged cells from our body.

How to design Drugs to shut off p53 mutated gene?

The famous Senator from Minnesota, Senator Hubert Humprey, died of Liver Cancer caused by the mutation on p53 gene. His illness was not identified for years. To those who are responsible for designing drugs to treat such diseases, I present my own work as an example. The human p53 gene is located on chromosome 17 which is made of 92 million nucleotide base pairs carrying 1,394 genes. P53 gene is made of 7,687,779 base pairs long. This gene encodes a tumor suppressor protein containing transcriptional activation. The specific mutation on p53 gene is located on the short arm of chromosome 17p13, consists of 11 exons coding for a nucleophosphatase-protein, which can bind to specific DNA sequences acting as a transcription factor. Today, the next generation of Sequencers, such as the Nanopore Sequencer could read a million nucleotides per second. Nanopore-type sequencers have been commercialized by Oxford Nanopore Technologies. Protein nanopores are arrayed on a membrane to detect changes in an electrical current when a DNA or an RNA molecule passes through the pore, permitting direct sequencing of the molecules.

By comparing p53 mutated gene sequence with Reference Sequence, we can easily identify the specific mutation on p53 genome responsible for causing the cancer. In my Lab, at the Drug Development Branch of the National Cancer Institute, at NIH, we used to find dyes to color tissues. Once a specific dye is found, we used to attach highly toxic Aziridines and Carbamate moieties to attack the tumor of that tissue. We have 220 tissues in our body, we can find a color for each tissue. Enormous number of color combinations are available. For example, with four nucleotides we get 64 nucleotide combinations. The Rainbow has seven colors, can you imagine how many color-combinations. are available. During summer holidays, with a small grant to high school students, we can easily find a color for all 220 tissues. Using Dinitrophenyl dye, my work above described how to design drugs by attaching Aziridine and Carbamate to shut off a gene responsible for causing

animal cancer (Walker Carcinoma-256 in Rats). and then using Quinone as a carrier for Aziridine and Carbamate how I made AZQ. By making AZQ (US Patent 4,233,215), I have demonstrated how to design drugs to shut off genes responsible for causing Glioblastoma, the brain cancer. It is the challenge for the next generation of scientists (my students) to activate the prodrug moieties derivatives of Aziridine and Carbamate to shut off mutated p53 genes to prevent cancers. One of the greatest challenges in designing drugs is to attack the DNA to shut off a gene. It was successfully carried out by Ross using highly toxic Nitrogen Mustard.

Using Nitrogen Mustard to design drugs to shut off a mutated gene?

Fitz Haber, a German Army officer, worked on the development of Chemicals as a Weapon of War. He was responsible for making deadly Nerve gases and Nitrogen Mustards. Before the WWI, he was honored with a Nobel Prize for capturing Nitrogen directly from the atmosphere for making Nitrate fertilizers by burning the element Magnesium in the air forming its Nitride. Upon hydrolysis, Nitride is converted to its Nitrate. Using this method, we could make unlimited amount fertilizer. Nitrate is also used for making explosive. Soon after the WWI, Haber was charged with a crime against humanity for releasing hundreds of cylinders of Chlorine gas on the Western front killing thousands of soldiers in the trenches. When Germany lost the war and Allied forces were looking for Haber. When they reached his residence, his son shot himself and his wife committed suicide. Haber went in hiding in Swiss Alps. After the War, German Government got his release as a part of the peace negotiations. Haber returned home to hero's welcome. Although he promised never to work on the chemical weapons again, secretly he continued to develop more lethal analogs of highly toxic chemicals like Nitrogen Mustards. It was Haber who first made the notorious Bis-dichloro-ethyl Methyl Amine. Because it smells like Mustard seeds, it is called as Nitrogen Mustard. During the next 20 years, before the beginning of the WWII, hundreds of more toxic analogs of Nitrogen Mustard were developed. The bad news is that they are highly toxic, and the good news is that they shut off genes.

Ross' Rationale for using War chemicals to treat Cancers:

Professor WCJ Ross of London University was the first person who used Nitrogen Mustard, a chemical weapon, to attack DNA for Cancer Treatment. 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 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.

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 for treating cancers such as Chlorambucil 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 Oral or Lung Cancers. [7., 8., 9., 10., 11., 12.]

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 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.

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 the 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 [13]. 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.

Nitrogen Mustard Shut off a gene by cross-linking both strands of DNA

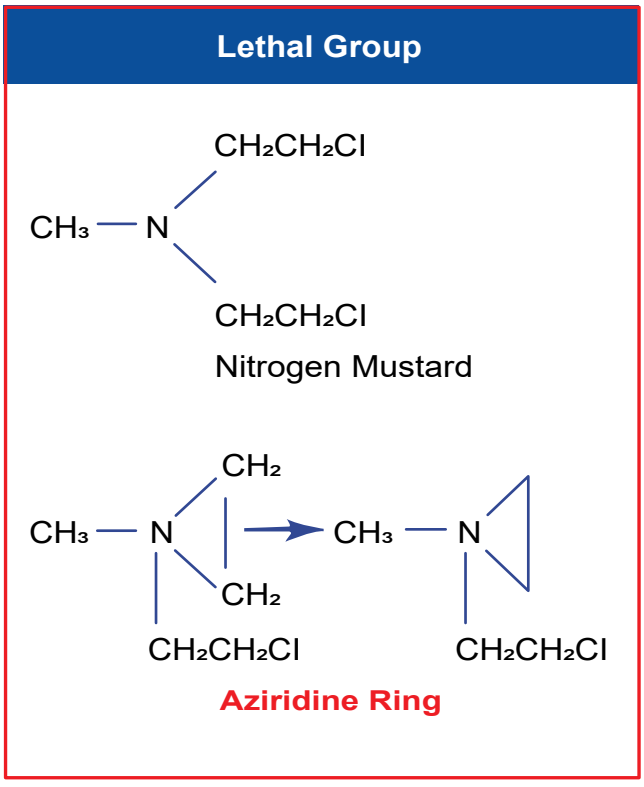
As I said above, I had made several dozens of analogs of Nitrogen Mustards for Professor Ross. I will describe how to make the Nitrogen Mustard by using Haber's crudest method. Haber reacted Methylamine with Ethylene oxide to make 2-bis dihydroxy ethyl methyl amine. It was chlorinated by heating with Phosphorus Penta Chloride in the Phosphoric Acid. If you noticed a faint smell of Mustard Seed, Congratulations,

you got Nitrogen Mustard; you cool 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 the solution to make its solid Hydrogen-Chloride salt. Nitrogen Mustard Hydrogen Chloride salt is separated. No matter how much 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 spectral line 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 cell to cancer cell gives a ratio of toxicity called the Chemotherapeutic Index (CI). The higher the ratio the more toxic the chemicals are to cancer cells. When tested against Walker Carcinoma 256 in Rats, most Nitrogen Mustards analogs cross-link both strands of DNA and give a CI of ten.

Shutting off a gene by binding to a single strand of DNA

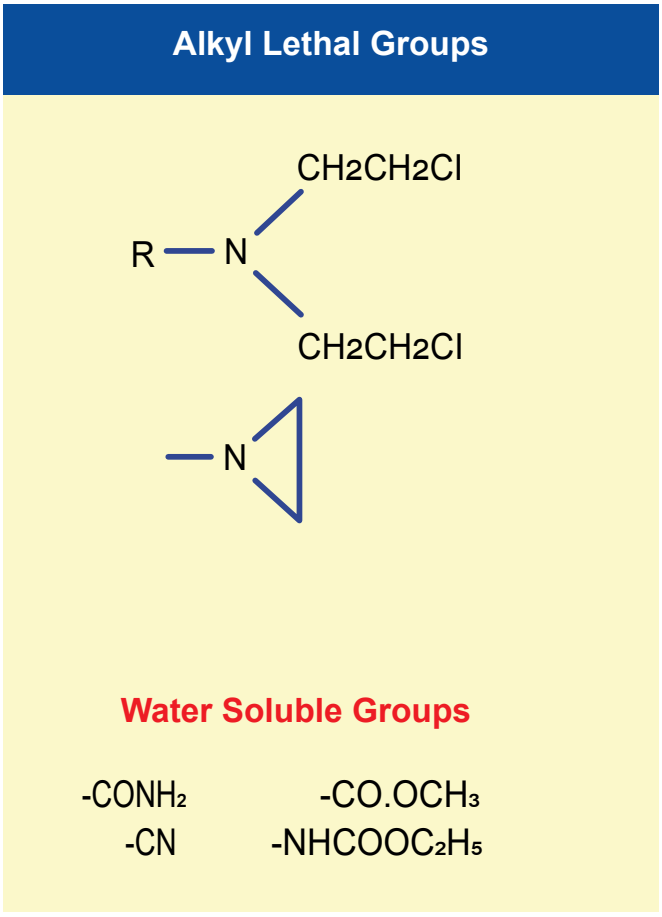
Aziridine Analogs as Anti-Cancer Agents serving as Pro-Drugs

A radiolabel study to understand the mechanism of action of Nitrogen Mustard 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 the 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 analog 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.



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.

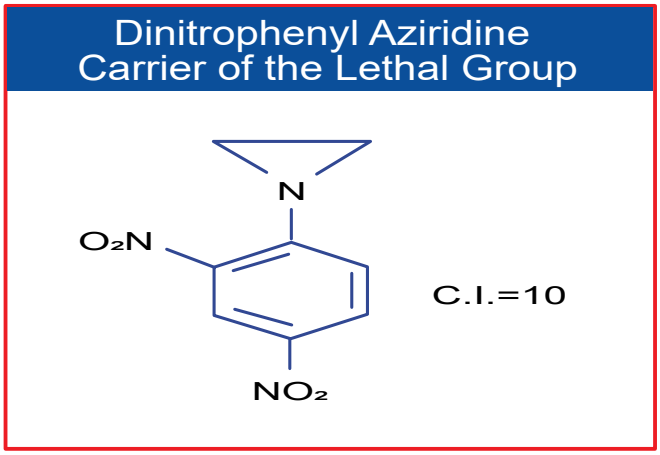
DNA Binding Lethal Groups

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 disrupts the Oxidative Phosphorylation (OXPHOS) of the ATP (Adenosine Triphosphate) which provides energy to perform all our body functions. To provide energy to 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.

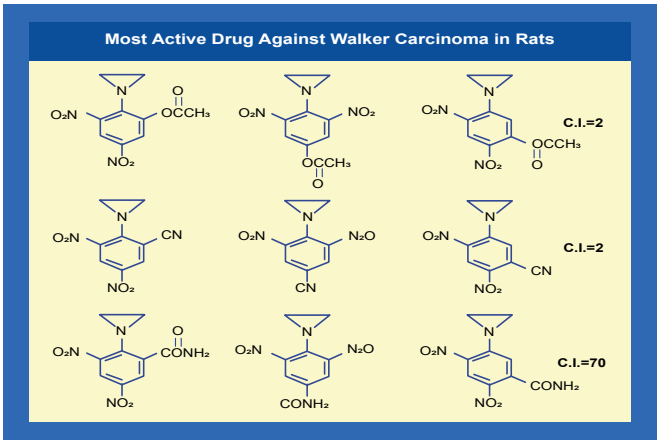
On further reexamination of the X-ray photographs of Dinitrophenyl Aziridine, it appeared that most of the radioactivity was concentrated at the injection site. Very little radioactivity was observed at the tumor site. It was obvious that we need to make derivatives of Dinitrophenyl Aziridine to move 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.



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 method. Ortho substituent always give 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 isomer 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:

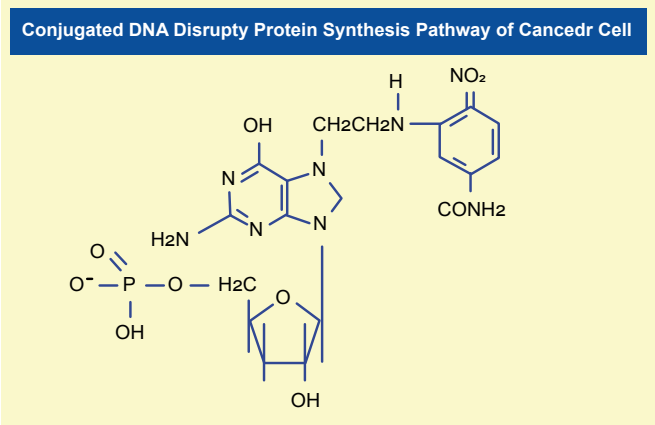
On the other hand, when the least water-soluble **Cyano-group** was attached to all three isomers of the 2,4-Dinitrophenyl aziridine compound as shown in the second line of the above chart, most of the compound stayed at the injection site. Only the last Cyano-derivative attached to DNA showed some anti-tumor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

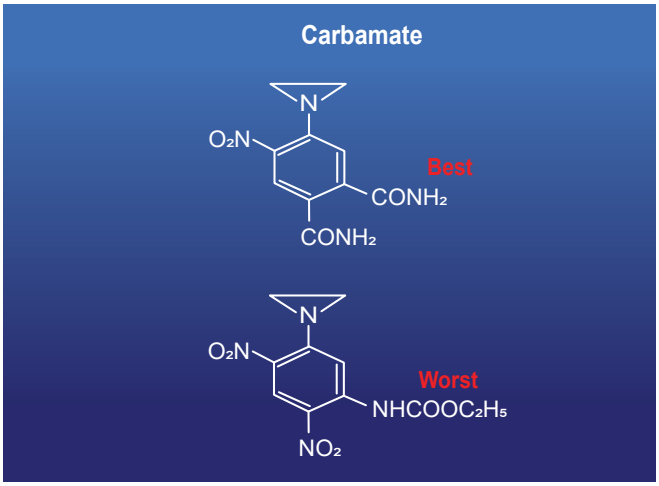
from the injection site to the tumor site. The drug 1-Aziridine, 2,4-dinitro, 5-benzamide (CB1954) showed the highest anti-tumor activity. It has a CI of seventy; it is seventy times more toxic to cancer cells, highest toxicity ever recorded against Walker Carcinoma 256 in Rats. [14., 15., 16.]

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 prodrug 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 show how CB1954 binds to a single stranded 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 with the Institute of Cancer Research (ICR) post-doctoral fellowship award to synthesize more analogs of CB1954. To improve drug delivery method, 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 so toxic that its Therapeutic Index could not be measured. We stop the work. Further work in London University was discontinued for safety reason.



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.

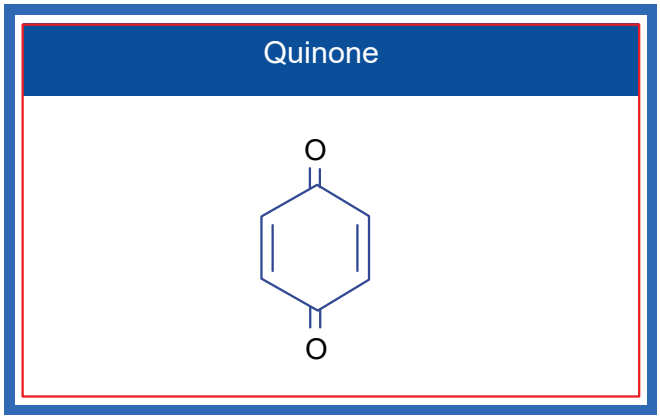
As a part of the inter-government agreement between UK and USA, all novel drugs developed in England were sent to the National Cancer Institute (NCI) in America for further screening. To translate animal work to human, I was invited to continue my work on the highly toxic Aziridine/Carbamate combination 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), USA. For making more Aziridine/Carbamates, I brought the idea from London University of attacking one strand of DNA using not only Aziridine, but also Carbamate without using the same dye Dinitro benzamide. My greatest challenge at NCI is to translate the animal work to humans.

In developing drugs for treatments, we poison bad DNA selectively. All poisons are a class of chemicals that attacks 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 chemical, 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 a cancerous breast; we still burn cancer cells by radiations; 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 as brutal and savage, but today that is all we have. We hope to develop new treatment for Breast Cancer. Hopes means never ever to give up.

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 is 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.

The Rational for Designing drugs to treat Glioblastoma, the human brain cancers:

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)

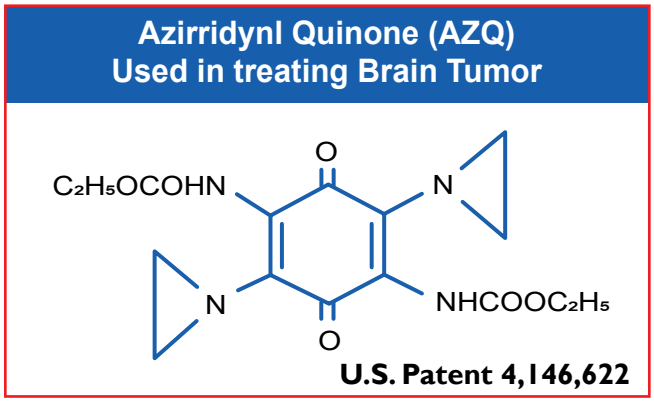
With the Quinone ring, I could introduce two Aziridine rings and two Carbamate moieties and could create havoc for Glioblastoma. Within three years, I made 45 analogs of Quinone. One of the Quinone carries two aziridines and two carbamate moieties which was highly toxic to Glioblastoma. The tumor stops growing and started shrinking. I named the Di-aziridine Dicarbamate Quinone, AZQ. My major concern was how toxic this compound would be to the 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 stop growing and start shrinking.

My drug AZQ is successful in treating experimental brain tumor 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 technique 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:

CI 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. 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 cancers, Glioblastomas. Literature search showed that AZQ is extensively studied as a pure drug and in combination with other anti-cancer drugs.



Single Strand DNA Binding Aziridine and Carbamate

As I said above, Glioblastomas, the brain cancers, 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 to DNA nucleotides by exposure to radiations, 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 represent 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) is also implicated in some forms of Glioblastomas.

All known Glioblastomas causing genes are located on five different chromosomes and carries 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 mega sequencing genome project, 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, and by making AZQ to treat human Glioblastoma, we have demonstrated that all bad genes can be shut off using Aziridine or Carbamate or both as attacking agents to shut off a gene. 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 to the 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)

What other cancers should we explore next?

Could I use the same rationale for treating Breast tumor?

Although BRCA1 gene located on Chromosome-17 (which is made of 92 million nucleotide bases carrying 1,394 genes) has been identified years ago, we wonder why it has been so difficult to treat Breast Cancer. By the time the Breast Cancer diagnosis is confirmed in a patient, the BRCA1 has accumulated more than three thousand mutations. Genotyping of the blood would also show that composition of many cells carrying mutated cell for creating secondary deposits. It is also believed that by the time Breast Cancer is confirmed, metastatic cancer cells have already been spread from liver lung on its way to brain. Since all other organs including breast and liver could be removed and replaced by breast implant except brain, I thought that protecting brain is utmost important treatment. Once AZQ is developed to protect the brain, I could focus on the Breast and Prostate Cancers.

Now, I found out that I could go even further by attaching more than four Aziridine and Carbamate moieties to both Male and Female Hormones. Radiolabeled studies showed that male hormone Testosterone has great affinity for female 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 attack the Breast and the Prostate cancer.

In a Breast tumor, within the start and stop codon, BRCA1 gene has captured over two hundred thousand nucleotide bases. The BRCA1 genes carries about three thousand mutations. These mutations are caused by radiations, chemical or environmental pollutants, viral infection or genetic inheritance. To attack the mutated nucleotides among the three thousand cells in BRCA1 gene, I could use male hormone, Testosterone, and bind multiple radio-labeled Aziridine and Carbamate ions to attack BRCA1 mutations. By using MRI, [19., 20] I could show how many radio-labeled nucleotides were bound to which mutations. Out of seventeen positions available for substitutions on Testosterone. There are only three positions that is 1,3 and 17 positions are available on Testosterone ring system. I could activate position 9 and 10 by reacting with Bromo-acetamide which introduce a Bromo ion on position 10 which could be dibrominated by Collidine to introduce a 9,10 double bond which I could further brominate to produce 9,10 dibromo compound. These bromo ion could be replaced by additional Aziridines or Carbamate ions. I could increase or decrease the number of Aziridine and Carbamate ions to get the maximum benefit by further brominating position 15 and 16 to introduce additional Aziridine and Carbamate moieties. Carl Djerassi C. Djerassi et al. J. Amer. Chem. Soc. 72. 4534 (1950)] 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-

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), 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-019-01/0). 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

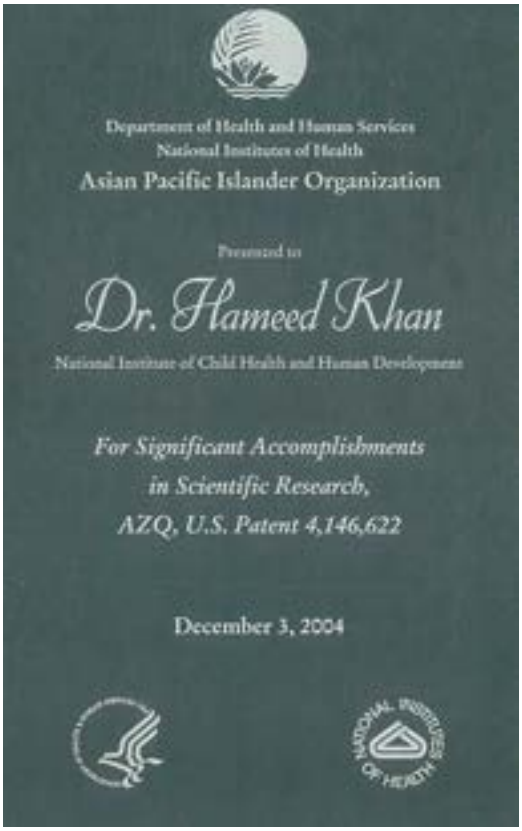


Exhibit # 2

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

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 Higness 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.

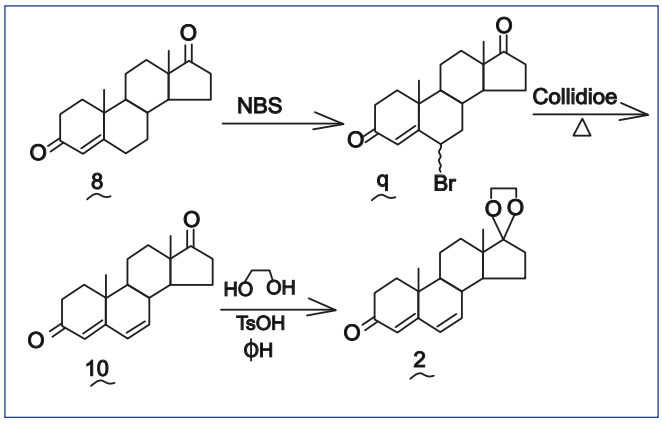
Exhibit # 4

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.

While Genome Center at NIH is supporting research on sequencing and mapping of the Genomes, my Institute NICHD was supporting research on Gene Markers associated with diseases

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, I could use the female hormone Estrogen and attach multiple Aziridine and Carbamate ions to attack Prostate tumor. Since there are seventeen positions available on Estrogen ring as well; again, I could increase or decrease the number of Aziridine and Carbamate ions to get the maximum benefit. Future generation of scientists (my students). [21-46] will use this method to develop drugs to treat all cancers.

Conclusion

As genome sequencing becomes cheaper and faster by Nanopore sequencer, we should be able to make enormous progress in prevention. For example, using the new editing technique of CRISPER cas-9, we should be able to wipe out Malaria. It is a mosquito-borne infection caused by a virus called Plasmodium Falciparum which is responsible for causing the disease. Malaria annually affects an estimated 247 million people worldwide and causes 619,000 deaths each year. Now, using CRISPER technology, the new generation of scientists want to wipe out Malaria from the face of the Earth. By using CRISPER, we can make female mosquitos sterile and release in the atmosphere. Within few generations, we could wipe out all mosquitos. There are critiques who say that we have no right to wipe out a species because it is harmful to humans. Not so long ago, we captured every Polio Virus and every Small Pox Virus sealed into a bottle and buried into a deep vault. These viruses are not dead and gone forever from the face of the Earth. They are waiting to become activated and alive if we wish. Those scientists were hailed as heroes. Now, our ethical principles have changed. As my former boss, Dr. Varmus used to say," New knowledge could create new problems, but knowledge is always superior to ignorance."

As the world population increased to eight billion, we add 100 million new individuals each year to take care. Time has come to consider the quality control of the population. Checking the genome sequence of the couple's parents and other family members and comparing their genomes with the Reference sequence data will help eliminate defected embryos and reduce the population of prisons, mental hospitals, and Asylums. You cannot live in the glorious serine past. We live in a polluted world. The Industrial Revolution has produced enormous amount of chemical and environmental pollution contributing to mutation in all living creatures. Radiations alone from fissionable material present a major cause of mutation. It is the responsibility of couple who wants to become parents to have healthy children by taking prevention before conception. In new Eugenic, it is not the authority, but the parents make the decision to bring healthy children into this world. They alone would decide if the childern they are bringing to this would be an acceptable member of the Human Society.

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

References

Ancient References that can be Googled on your cell phone are removed.

1. Watson, J. D., & Crick, F. H. C. (1953). A structure for deoxyribose nucleic acid. Nature, 171, 737–738.

2. Lander ES, Linton LM (2001) The Impact of Sequencing Human Genome on Genomic Food Nature 409: 934-941.

3. Lander ES, Linton LM (2001) Initial sequencing and analysis of the human genome. Nature 409: 660-921.

4. International Human Genome Sequencing Consortium (2004) Finishing the euchromatic sequence of the human genome. Nature 431: 931-945.

5. Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, et al. (2005) Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 438: 803-810.

6. Shendure J, Balasubramanian S, Church GM, Gilbert W, Rogers J, et al. (2017) DNA sequencing at 40: past, present and future. Nature 550: 345-353.

7. Ross WCJ (2015) Drug Information: Description, User Reviews, Drug Side Effects, Interactions – Prescribing Information at RxLis Chlorambucil. Journal of the American Chemical Society 136: 5860-5863.

8. Ross WCJ (1953) The Chemistry of Cytotoxic Alkylating Agents In Advances in Cancer Academic Press 397-449.

9. Ross WCJ (1962) Biological Alkylating Agents. Butterworth, London

10. Ross WCJ (1949) Journal of Chemical Society. 183.

11. Ross WCJ (1950) J. Chem. Soc. 2257.

12. Ross WCJ, Mitchley BCV (1964) Ann. Rep. Brit. Empire Cancer Campn 42: 70.

13. Ross WCJ (2007) Melphalan and prednisone plus thalidomide versus. Melphalan Lancet 370: 1209-1218.

14. Cobb LM, Connors TA, Elson LA, Khan AH, Mitchley BCV, et al. (1969) 2,4-Dinitro-5-Ethyleneiminobenzamide (CB 1954): A Potent and Selective Inhibitor of the Growth of the Walker Carcinoma 256. BIOCHEMICAL PHARMACOLOGY col 18: 1519-1527.

15. Khan AH, Ross WCJ (1969/70) Tumour-Growth Inhibitory Nitrophenylaziridines and related compounds: Structure-Activity Relationships PART I. CHEM.-BIOL INTERACTIONS 1: 27-47.

16. Khan AH, Ross WCJ (1971/72) Tumour-Growth Inhibitory Nitrophenylaziridines and related compounds: StructureActivity Relationships. CHEM.-BIOL INTERACTIONS 4: 11-22.

17. Hameed Khan A, John Driscoll (1976) Potential Central Nervous System Antitumor Agents: Aziridinylbenzoquinones. PART I. JOURNAL OF MEDICINAL CHEMISTRY 19: 313-317.

18. Chou Ed, Hameed Khan A, John Driscoll (1976) Potential Central Nervous System Antitumor Agents: Aziridinylbenzoquinones. PART II. JOURNAL OF MEDICINAL CHEMISTRY 19: 1302.

19. Hameed Khan A (2021) The Impact of Diagnostic MRI on the Early Detection of Lethal Genes in Human Genome and to Develop Genomic Medicine to Treat Brain Cancers. J Med - Clin Res & Rev 5: 1-9.

20. Hameed AK (2021) The Impact of Diagnostic MRI on the Early Detection of Lethal Genes in Human Genome and to Develop Genomic Medicine to Treat Brain Cancers. J Med - Clin Res & Rev 5: 1-9.

21. Hameed Khan (2018) The Impact of Sequencing Human Genome on Drug Design to Treat Oral Cancer. IntechOpen https://www.intechopen.com/chapters/63395

22. Ala-Eddin Al Moustafa (2017) Development of Oral Cancer – Risk Factors and Prevention Strategies. Springer https://link.springer.com/book/10.1007/978-3-319-48054-1

23. Hameed AK (2021) The Impact of Diagnostic MRI on the Early Detection of Lethal Genes in Human Genome and to Develop Genomic Medicine to Treat Brain Cancers. Clin Res & 5: 1-9.

24. Berhardt LV (2021) e-Book, The Impact of Sequencing Human Genome on Genomic Medicine and the Discovery of AZQ (US Patent 4,146,622) Specifically Designed to shut off genes that cause Brain Cancer Chapter I. NOVA Medicine and Health 180 1- 63.

25. Abdul Hameed Khan (2021) The Rational Drug Design to Treat Cancers: Drug Design - Novel Advances in the Omics Field and Applications. London 117.

26. Hameed Khan (2021) The Impact of Sequencing Human Genome on Genomic Food & Medicine International Journal of Genetics and Genomics 9: 6-19.

27. Hameed Khan (2021) The Impact of Sequencing Genomes on The Human Longevity Project. J Med - Clin Res & Rev 5: 1-12.

28. Hameed AK (2021) The Impact of Diagnostic MRI on the Early Detection of Lethal Genes in Human Genome and to Develop Genomic Medicine to Treat Brain Cancers. J Med - Clin Res & Rev 5: 1-9.

29. Hameed AK (2021) The Impact of Sequencing Genomes on The Human Longevity Project. J Med - Clin Res & Rev 5: 1-12.

30. Hameed Khan (2021) The Impact of Sequencing Human Genome on Genomic Food & Medicine. International Journal of Genetics and Genomics 9: 6-19.

31. Hameed Khan (2021) ADVANCES IN MEDICINE AND BIOLOGY, VOLUME 180, The Impact of Sequencing Human Genome on Genomic Medicine and the Discovery of AZQ https://novapublishers.com/shop/advances-in-medicineand-biology-volume-180/

32. Hameed Khan A (2021) The Rational Drug Design to Treat Cancers Chapter 5 Drug Design - Novel Advances in the Omics Field and Applications Page 95-115.

33. Abbreviated Key Title: EAS J Biotechnol Genet (Online) Published by East African Scholars Publisher, Kenya https:// www.ipindexing.com/journal-details/EAS-Journal-ofBiotechnology-and-Genetics/1236

34. Hameed Khan (2021) Genomic Medicine: Using Genetic Make-up of the Human Genome, Genomic Medicine: Using Genetic Make-up of the Human Genome, AZQ was Designed to Treat Glioblastoma, the Brain Tumor. Nov Appro in Can Study https://crimsonpublishers.com/nacs/fulltext/NACS.000634.php

35. Hameed Khan A (2021) The Impact of Sequencing Human Genome on Cancer Chemotherapy. J Can Res Rev Rep 3: 1-10.

36. Hameed Khan A (2021) The Impact of Sequencing Genomes on the Understanding of the Origin of Life on Earth. Biomed J Sci & Tech Res 40: 32008-32022.

37. Hameed Khan A (2022) The Impact of Sequencing Human Genome on the Genetically Engineered Life. J. Cancer Research and Cellular Therapeutics. 6(1); DOI:10.31579/2640-1053/102.

38. Hameed Khan A (2022) Sequencing the Human Genome to Study the correlation between Genes and Violence. Horizon publishers

39. Hameed Khan A (2022) The Impact of Sequencing Human Genome on the 2nd Genesis on Earth, Journal of Current Trends in Agriculture, Environment and Sustainability. Katalyst publisher https://www.youtube.com/ watch?v=z4BGzLNSgpY

40. Hameed Khan A (2022) Assessment of Human Genome Sequencing on the New-World Order. Research Aspects in Biological Science 4: 978-993.

41. Hameed Khan A (2022) Assessment of Human Genome Sequencing on the New-World Order. Research Aspects in Biological Science 4: 978-993.

42. Hameed Khan A (2022) The Impact of Sequencing Human Genome on Novel Drug Delivery Method to Treat Cancers. International Journal of Genetics and Genomics 10: 64-78.

43. Hameed Khan A (2022) The Impact of Sequencing Human Genome on New Eugenic., The Impact of Sequencing Human Genome on the 2nd Genesis on Earth. J Cur Tre Agri Envi Sust 5: 1-21.

44. Hameed Khan A (2023) The Impact of Sequencing Human Genome on Idiopathic Diseases. Biomedical Sciences 5: 2421-2437.

45. Hameed Khan A (2023) The Impact of Sequencing Human Genome on Darwinian Evolution. Khan H. J Can Ther Res 3: 1-50.

46. A Hameed Khan (2024) The Impact of Sequencing Human Genome is not only to Treat all Genetic Diseases, But also to Extend Human Life to Travel into Deep Space in Search of Exo-Planets to Protect, Preserve and Spread Human Intelligence Across the Universe. Journal of Cancer Research Reviews & Reports. SRC/JCRR-196. DOI: doi.org/10.47363/JCRR/2024(6)186