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Review Article

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The Impact of Sequencing Human Genome on Mitochondrial Disorder

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ABSTRACT

The purpose of this abstract is to study inherited mitochondrial diseases (MD) for which effective treatments do not exist. The purpose of Sequencing Human Genome is to Diagnose a mutated gene and then Prevent and Treat a disease. The fact that the majority of pathogenic mutations are found in coding regions; the coding gene regions, the 'exome', comprise approximately 1.6% of the human genome. The fastest way to identify a disease-causing mutation is from the exome sequencing alone. Our body carries two genomes, a human genome and a mitochondrial genome. While our genome is located on liner chromosomes, Mitochondrial genome has the circular chromosome found inside the cellular organelle. Located in the cytoplasm, mitochondria are the site of the cell's energy production and other metabolic functions. Mitochondrial diseases are caused by pathogenic mutations that directly affect the energy metabolism that takes place inside mitochondria in the process of Oxidative Phosphorylation (OXPHOS). It involves large respiratory chain (RC) enzyme complexes, and hence are known collectively as RC diseases. Sequencing is the best diagnostic technique and it helps us identifying the mutation responsible for causing the disease. Once the serious mitochondrial disorder is confirmed by sequencing, it is the parents not the authority who decide if the fetus survives or not. If the parents decide to keep the child alive, the next step is either to have three-parent children or to develop treatments of the disorder either by gene therapy or by drug therapy. Compare to human genome, Mitochondrial genome is very small. 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 like Aziridine and Carbamate to shut off acid producing Latic Acidosis mitochondrial genes without attacking the normal mitochondria.

Keywords: Mitochondrial Disorder, Oxidative Phosphorylation, RC Diseases, Glioblastoma. BBB, Aziridine, Carbamate, AZQ

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.

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.

Ancient References That Can be Googled on Your Cell Phone are Removed

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Introduction

The Origin of Mitochondria

The origin of Mitochondria began soon after the Pre-Cambrian era: The geologic era before the Cambrian was called the Ediacaran, lasting from about 635 to 542 million years. Scientists often characterize this era as an "experimental" phase in the evolution of complex life on Earth. For most of the nearly 4 billion years that life has existed on Earth, evolution produced little change beyond bacteria, plankton, and multi-celled algae. With an environment devoid of oxygen and high in Methane and Carbon dioxide, during Pre Cambrian era, for much of its history, Earth would not have been a welcoming place for multicellular life. The earliest life forms we know of were microscopic organisms (microbes) that left impressions of their presence as fossil on the surface of rocks about 3.7 billion years old rocks.

But beginning about 600 million years ago climate change brought rise in temperature resulting production of Oxygen causing Cambrian Explosion by bringing changes in species from asexual to sexual reproduction which brought dramatic changes in the genome of living species. Changes occurred from Procaryote to Eukaryote to multicellular organism; the fossil record show the more rapid change. Around 530 million years ago, a wide variety of animals appeared onto the evolutionary scene in an event known as the Cambrian explosion. With the arrival of Blue Green Algae, the photosynthetic apparatus was set up. The chlorophyll carrying algae, conducted photosynthesis on massive scale. The Blue Green Algae's job was to absorb Carbon dioxide from the atmosphere and in the presence of sunlight and water, carry out photosynthesis converting Carbon dioxide to its food Carbohydrate and releasing Oxygen as its by-product.

For billions of years, Blue Green Algae must have carpeted the entire planet's surface absorbing Carbon dioxide and pumping Oxygen. Aerobic life appeared. In perhaps as few as 10 million years ago, marine animals evolved most of the basic body forms that we observe in modern living creatures. The Cambrian Explosion saw an incredible diversity of life emerging, including many major animal groups alive today. Among them were the chordates, to which vertebrates (animals with backbones) such as humans belong. A steep rise in Oxygen which could have had a profound effect on the climate and the evolution of life on Earth. Oxygen is highly toxic to the anaerobic life. With the arrival of massive amount of Oxygen, (20% Oxygen and about 80% Nitrogen the primitive anaerobic life forms disappeared.

A new bacterial life form called Mitochondria appeared which were once prokaryotes themselves (their free-floating chromosomes have no nuclear membrane). In the depths of history, a free-living bacterium was engulfed by a larger cell and was neither digested by enzymes nor destroyed. Instead, it was harbored within the cell. It forged a unique and fateful symbiotic partnership with its host cell, eventually becoming the Mitochondria of today. Mitochondria contain their own genome separate from the host cell nuclear genome and they are evolved from prokaryotic ancestors some two billion years ago. They provide the main source of the cell's energy supply and are involved in such important processes as apoptosis, mitochondrial diseases and aging. Mitochondria originated by permanent enslavement of purple non-Sulphur bacteria. These endosymbionts cells became organelles through the origin of complex protein-import machinery and insertion into their inner membranes of protein carriers for extracting energy for the host. More precisely, scientists believe that mitochondria originated when primitive anerobic prokaryotic bacteria were captured by aerobic eukaryotes, then permanently incorporated into their structure. Mitochondria thrive in human because it was captured by Eucaryotic. In plants such as Blue Green algae, Chloroplasts perform photosynthesis producing Carbohydrate and more Oxygen.

Some scientists (who believe Mitochondria provides ATP as a source of energy) believe that during Precambrian era about 450 million years ago when multicellular organisms were evolved, a single procaryotic (a cell with no nuclear member to contain chromosomes) was captured by a multicellular Eucaryotic organism with a nuclear member accepted to house Procaryotic chromosomes in its cytoplasm. The multicellular organisms have agreed to serve as a host. It provides the procaryote free food and shelter outside the nucleus in the cytoplasm. In return, the procaryote agreed to provide unlimited amount of energy to the host organism by breaking energy rich Adenosine Triphosphate (ATP). During host cell division, ATP energy rich phosphate bond is broken down to Adenosine Diphosphate (ADP) releasing phosphate bond energy which is further broken down to produce more energy for the host cell by breaking down to Adenosine monophosphate (AMP). The enzyme phospho-kinase converts AMP back to ATP by incorporating inorganic phosphorus by the enzyme Phosphokinase, both species thrived and evolution proceeded rapidly.

Over the eons, mitochondria coevolved with their hosts as organelles, but the former bacteria never fully jettisoned their genes. Today, mitochondria retained 37 genes to code for 13 proteins, leaving the remaining 99% of the proteins it needs to the host cell nuclear genome. But where there are genes, there is replication and errors are bound to occur. Mutations in mitochondria- associated genes lead to mitochondrial diseases. These multisystemic disorders can impact any Organ supply to those organs with the highest energy demands, such as the brain and heart, tend to be the most affected by Mitochondrial diseases.

Human egg and sperm are loaded with mitochondria in the cytoplasm outside their nucleus. During conception, when a sperm fuses into the egg, the tail of the sperm containing mitochondria is dropped off. The fetus carries only maternal mitochondria (mtDNA). All father's mitochondrial diseases are passed down from mother to children. The discovery of mitochondrial DNA (mtDNA) mutations in diseases was indeed seminal, demonstrating that the increased incidence of maternal inheritance in mitochondrial disorders could be explained by defects in the exclusively maternally inherited mtDNA. To understand how mitochondrial diseases are inherited, it is important to know that there are two types of genes essential to mitochondria. The first type is housed within the nucleus a compartment within our cells that contains most of our genetic material, or DNA. The second type resides exclusively within DNA contained inside the mitochondria. Mutations in either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) can cause mitochondrial disease. Mutations in any of the more than 1,000 nuclear genes encoding a mitochondrial protein can ostensibly cause a mitochondrial disorder. In mitochondrial diseases, the mitochondria cannot

efficiently turn sugar and oxygen into energy, so the cells do not work correctly. There are many types of mitochondrial disease, and they can affect different parts of the body at different times of life. These mutations can affect the brain, kidneys, muscles, heart, eyes, ears, and including Poor growth. Additional problems include loss of muscle coordination, muscle weakness, Neurological problems, including seizures. Autism spectrum disorder, represented by a variety of ASD characteristics, Visual and/or hearing problems. Developmental delays, learning disabilities, heart, liver or kidney disease. Children with MD have a short life expectancy. In a study in 221 children with mitochondrial disease, 14% died within three to nine years after diagnosis. Five patients lived less than three years, and three patients lived longer than nine years. As I said above, one of the most important functions of mitochondria obviously include oxidative phosphorylation to produce cellular ATP, but they also have other important roles such as in ion homeostasis, in several metabolic pathways, in apoptosis that is in programmed cell death, and in ROS production and consumption.

Our genes are units of inheritance and carry instructions to make proteins and when the proteins fold, they become reactive and carry out a specific function. Hundreds of proteins interact to make a cell and millions of cells interact to make a tissue. Hundreds of tissues interact to make an organ and several organs interact to make a human being. We carry in our body 220 different tissues. The instructions to make tissues are written in our genes. A defected tissue could be identified by looking at the mutation in the genes. We can prevent diseases at a very early stage of our lives. By sequencing a fertilized egg, the genotype, we could identify the mutations responsible for future diseases in tissues, the phenotype. If a patient has a family history of a specific disease, to prevent future generation from inheriting the disease, it is best advice for such families to have conception by in vitro fertilization after making sure that the fertilized egg is free from all abnormal mutations responsible for causing the disease.

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

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

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Genes are the unit of inheritance. As I said above, out of four-letter text, that is A-T and G-C, and three letters code for an amino acid called the Codon. The starting Codon in a gene is the Codon AUG (instead of T nucleotide, we use U nucleotide because Thiamin is converted to more water-soluble Uracil) which codes for amino acid Methionine. Long chain of DNA synthesis begins. The starting Codon is followed by a series of hundreds of Codons which codes for different amino acids in different species. There are three Stop Codons and they are AUG, UGG, and UGA. Once the stop codons appear, DNA synthesis stops. Bacteria and Viruses have short codon chain. The longest chain is in a gene of Ducharme Muscular Dystrophy, a neurological disease whose chain extends to two and a half million codons. Once a gene is identified, using Restriction Enzymes, like EcoR1, which serves as molecular scissor, we can cut, paste and copy all genes individually making a Restriction Site map. Once a single gene is isolated; either from nDNA or mit DNA, we could compare the sequence of this gene with either Reference Seqence genome or the Thousand Genome Project to identify abnormal mutation responsible for causing the MD, we could design drugs to shut off that gene. Sequencing is like extracting Gold from its Ore.

Today, each human cell carries thousands of mitochondria providing all the energy we need to survive our entire life. Some of those mitochondria carry mutated nucleotides either provided by host cell genome or due to exposure of mitochondrial genome either to radiations, chemical environmental pollutions, viral infection or genetic inheritance. Our challenge is to identify, prevent and treat the mutations before it spreads causing sever mitochondrial illnesses.

As I said above, sequencing the patient's genome and comparing with the normal genome (Reference Sequence) provides the best method of diagnosis. It is easier with MD because, Mitochondrial genome is a closed circular double-stranded molecule approximately 16.6 kb in length. It contains 37 genes, 13 of which encode core structural components of the OXPHOS complexes, as well as 22 tRNAs and two rRNAs required for their synthesis. On the other hand, the human genome is made of six billion four handed million nucleotide base pairs carrying 24,000 genes located on 46 chromosomes. To understand the origin of life and the origin of diseases, it was considered absolutely essential to read our entire book of life, our genome, word by word, sentence by sentence chapter by chapter all forty-six-volume (chromosomes) consisting of six billion four hundred million letters (nucleotides) and also to determine the number and the order in which these letters are arranged (sequencing). By comparing their genomes with Reference Sequence, we can identify mutations responsible for causing the diseases.

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 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. In every cell of our body, we carry sixteen thousand good genes, six thousand mutated (bad) genes responsible for six thousand diseases and two thousand Pseudogenes that have lost their functions, during evolutionary time.

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. 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 [2-6].

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 isolated from a gene located in Pancreas was harvested in large scale in bacteria. 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 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 industrial scale such as the production Insulin to treat 300 million of diabetic 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 is 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.

The best advice for those couples who have a family history of long-term illnesses 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 are not permitted at this time. 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 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 a disease, 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 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 or bacteria and harvest them or move the gene from species to species. As I said above, we can harvest good genes to produce large scale protein such as Insulins to treat diabetics or design drugs to shut of bad genes to treat diseases such as cancers.

Three Parents Babies

As I said above, human body carries a second genomes besides Human Genome, there is also a microbial genome captured millions of years ago called the Mitochondrial genome. Mitochondria live in human cytoplasm in a symbiotic relationship with normal host cells. During conception, when mother's egg is fertilized by father's sperm, the tail of the sperm is dropped off and father's Mitochondria are lost. We inherit only mother's Mitochondria. Any mutations in the Mitochondrial genome, could cause sever diseases in the infants. Congenital lactic acidosis is a rare disease caused by mutations in mitochondrial DNA (mtDNA) that affect the ability of cells to use energy and cause too much lactic acid to build up in the body, a condition called lactic acidosis. Genetic defects in the pyruvate dehydrogenase complex are also responsible for the most common causes of primary Lactic Acidosis in children. Pyruvate dehydrogenase complex (PDC) deficiency is a genetic mitochondrial disease of carbohydrate metabolism that is due to a mutation in Human nuclear DNA (nDNA). It is generally considered to be the most common cause of biochemically proven cases of congenital Lactic Acidosis. Most cases are caused by mutation in the E1-alpha subunit gene on the X chromosome resulting in Pyruvate dehydrogenase E1-alpha deficiency. Congenital Lactic Acidosis (CLA) is a rare condition that is mainly due to a range of inborn errors of metabolism that result in defective mitochondrial function. Lactic acidosis results from the accumulation of lactate and protons in body fluids. Often, mitochondrial disease patients suffer from a condition known as Lactic Acidosis which is an increase in lactic acid concentration in the body. Lactate and pyruvate are acids found in the mitochondria. Once any of those above mitochondrial mutations are confirmed by sequencing, the safest way to have children is by in vitro fertilization. During in vitro fertilization, if mutation is discovered in Mitochondria, it can either be discarded or could use a healthy Mitochondria carrying egg from a second mother to prevent the transmission of the disease. This means the baby has three genetic parents: the father who supplies the sperm, the original mother who supplies both womb and the egg nucleus carrying mutated mitochondria, and an anonymous donor who supplies healthy mitochondria. Of these, the mitochondrial DNA is by far makes the smallest contribution. Mitochondrial donation offers women with disease free mitochondria an opportunity to have healthy, genetically related children. Some parents are against mitochondrial donation and their objections include safety, the creation of three-parent babies, ethical issues, impact on identity, implications for society, definitions of genetic modification and reproductive choice. In spite of all these concerns, the British government considered the donation process is safe and approved the donation of an egg with

healthy mitochondria. A third parents' baby was born in England and lives a normal life. To answer the American concerns, we need new ethical guidelines based on modern science.

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 disease. 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 disease. The next step is to shut off that gene either by gene therapy or drug therapy.

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. 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.

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.

How to Design Drugs to Shut off a Mutated Gene?

Historical Background for Using Nitrogen Mustard for Treating Cancer

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 [7-12].

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.

Synthesis of Nitrogen Mustard as Anti-Cancer Drugs

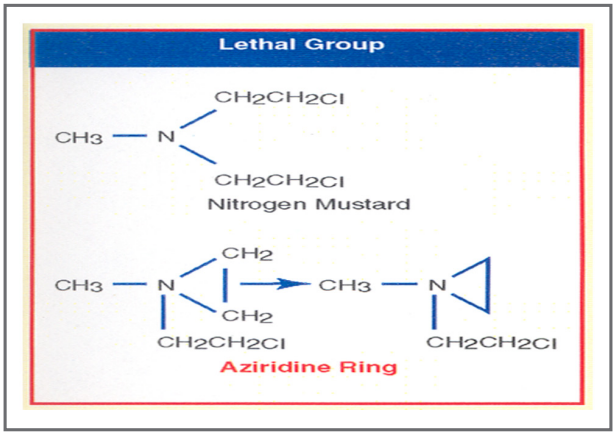
Nitrogen Mustard Shut off a Gene by Cross-linking Both Strands of DNA

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

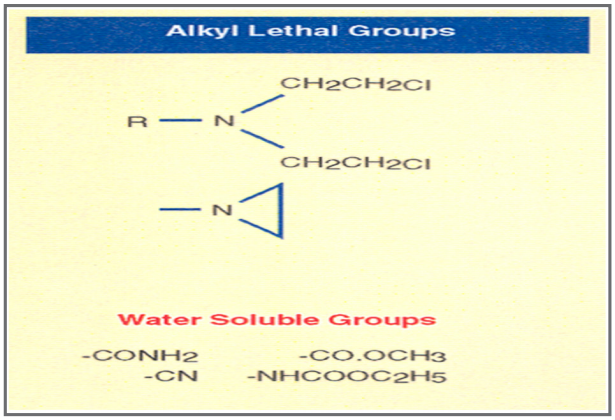
Aziridine Analogs as Anti-Cancer Agents Serving as Pro-Drugs

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.

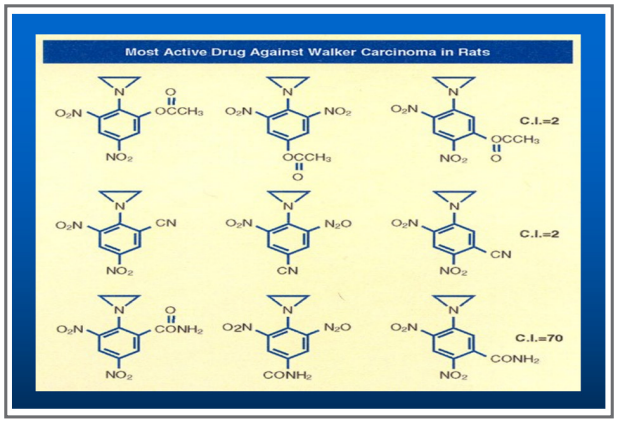
Further reexamination of the X-ray photographs of Dinitrophenyl Aziridine it appeared that most of the radioactivity was concentrated at the injection site. Very little radioactivity was observed at the tumor site. It was obvious that we need to make derivatives of Dinitrophenyl Aziridine to move the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

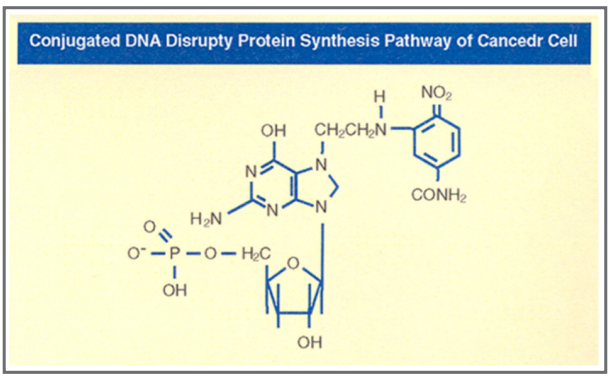
The Least Water-Soluble Substituent

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

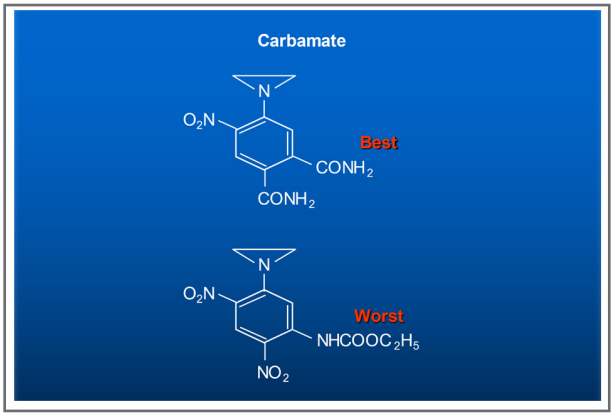
The last line of the above chart showed that the first two **Amido groups** were sterically hindered and did not bind to DNA and showed no biological activity, but the last compound presents the perfect drug delivery method. The entire drug was delivered from the injection site to the tumor site. The 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 [14-16].

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.

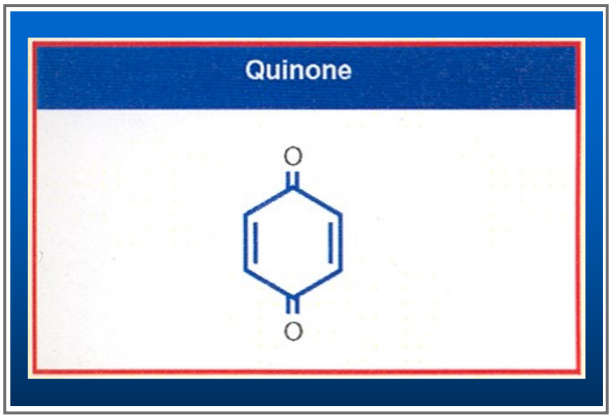
As a part of the inter-government agreement between UK and USA, all novel drugs developed in England were sent to the National Cancer Institute (NCI) in America for further screening. To translate animal work to human, I was invited to continue my work on the highly toxic Aziridine/Carbamate combination 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 serves as prodrugs that is they remain inactive in the basic and neutral media. They become activated only in the presence of acid produced by cancer cells.

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)

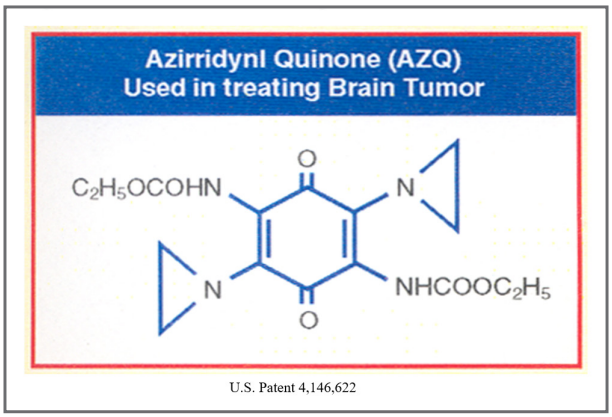
With the Quinone ring, I could introduce two Aziridine rings and two Carbamate moieties and could create havoc for Glioblastoma. Within three years, I made 45 analogs of Quinone. One of the Quinone carries two aziridines and two carbamate moieties which was highly toxic to Glioblastoma. The tumor stops 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

I decided to use Quinone moiety as a carrier for Aziridine rings to attack Glioblastomas. By introducing an additional Carbamate moiety, I could increase its toxicity several folds. I planned to use this rational to translate animal work to human by introducing multiple Aziridine and Carbamate moieties to the Quinone to test against Glioblastomas in humans. 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.



U.S. Patent 4,146,622

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 sequencing 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 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.

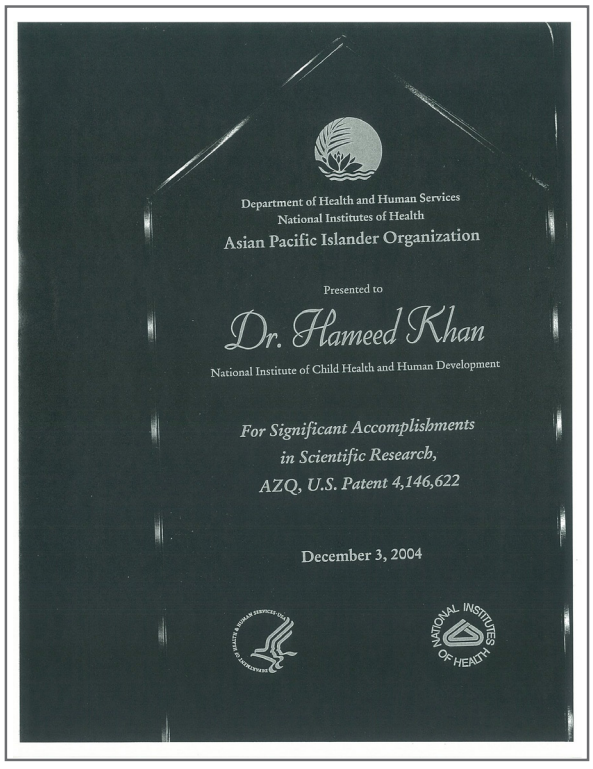
One of the greatest challenges of nanotechnology is to seek out the very first abnormal cell in the presence of billions of normal cells of our brain and shut off the genes before it spread. I worked on this assignment for about a quarter of a century; conducted over 500 experiments which resulted in 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)

Exhibit # 1

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

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



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

The Royals of Travancore

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

Exhibit # 4

Gold Medal for Dr. Khan

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

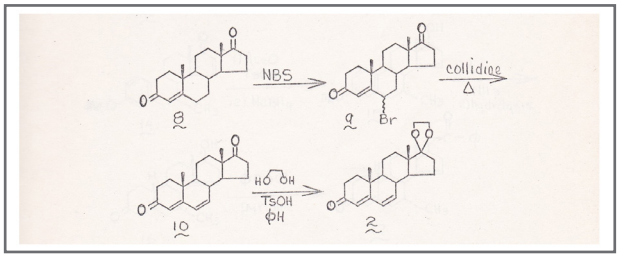
While Genome Center at NIH is supporting research on sequencing and mapping of the Genomes, my Institute NICHD was supporting research on Gene Markers associated with diseases.

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, I could show how many radio-labeled nucleotides were bound to which mutations [19,20]. 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-brominated by Collidine to introduce a 9,10 double bond which we could further brominate to produce 9,10 dibromo compound [21]. These bromo ion could be replaced by additional Aziridines or Carbamate ions. We could increase or decrease the number of Aziridine and Carbamate ions to get maximum benefit by further brominating position 15 and 16 to introduce additional Aziridine and Carbamate moieties.

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

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) [22-46]. will use this method to develop drugs to treat all cancers.

How to Shut off Lactic Acidosis Gene to Treat Mitochondrial Disorder

The most recognized laboratory abnormality in patients with mitochondrial disorders is the presence of Latic acidosis. The

overproduction of Lactic acid in Lactic acidosis is due to Dysfunction in the electron transport chain causes decreased production of adenosine triphosphate (ATP). Low adenosine triphosphate levels result in an up-regulation of glycolysis, leading to an overproduction of Lactic Acid. Most causes of congenital lactic acidosis are due to genetic mitochondrial enzyme deficiencies. SLSJ congenital lactic acidosis is caused by two types of mutations in the LRPPRC gene (2p21). The mutated genes responsible for the lactic acidosis could be shut off by using specific dyes responsible for coloring the mutated genes. The dyes are used as a carrier for transporting acid sensitive moieties such as Aziridine or Carbamate which could act as prodrug moieties. In the presence of Lactic Acid, the prodrug Aziridine and Carbamate are broken down releasing highly reactive Carbonium ions which attack the mutated nucleotide of MD and may shut off the genes.

By making (CB1954) Aziridine, 2.4-dinitronbenzamide tested in experimental animal tumors in Rats and (AZQ) Diaziridine-Quinone-Dicarbamate in Glioblastoma in Humans, I have demonstrated (US Patent 4,233,215) that the prodrug Aziridine and Carbamate derivative could be activated in the presence of Lactic Acid generating Carbonium ions shutting off genes. Similar rational could be used to treat at least one mitochondrial disorder Lactic Acidosis.

Conclusion

This lecture attempts to provide guidance to young couple as to what precautions to take and what questions to ask before they decide to become parents. Do you want to know your genetic make-up? There might be bad news. There might be a mutation for mitochondrial disease. I would like to see my sequence because I would prepare myself for future health problems. Accordingly, I can plan my future; what I should do, what I should not do which include financial planning for dependence; medical planning for oneself and family members etc. are essential precautions I must make. It is an individual choice. As a pregnant mother, do you want to look at the genetic make-up of yourself and compare with your future children? Do you have a family history of serious illnesses? Find out from your parents and grandparents. Are you a carrier of a horrendous illness that you could pass on to your children? Are your parents closely related? Are you a carrier and if your spouse also carries the same defected complimentary copy of the mutated gene? According to Gregor Mandel children from such closely related parents is likely to have one in fourth who will become sick and will come down with a horrendous disease. Sequencing the genomes of all three generations that is your parents, yourself and your soon to be born child and comparing their genomes of each with the Reference Sequencing will save you from the lifetime of sufferings. The next generation of Nanopore Sequencers will sequence your genome cheaper, faster, and with extreme accuracy and precision. You should know that the next generation of children will grow up in a fiercely competitive society. There are eight billion people live on Earth today and we are adding a 100 million children each year. In this rat race, children even with minor genetic defects will be left behind. (It does not matter if you are a prolife or prochoice, abortions will disastrously effect women's health. I oppose abortions at all cost. In order to have healthy baby, I recommend sequencing egg and sperm before conception. To produce healthy member of society, I also recommend conception by in vitro fertilization. Checking the genome sequence of the couple's parents and other family members and comparing them with Reference sequence will 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 chemicals and environmental pollution contributing mutation in all living creatures. Exposure to radiations from fissionable material present major cause of mutations. 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. Parents alone would decide if the child they are bringing to this would be an acceptable member of the Human Society.

The ideas expressed in this article are mine and do not represent NIH Policy

References

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

1. Watson JD, Crick FHC. A structure for deoxyribose nucleic acid. Nature. 1953. 171: 737-738.
2. Hameed Khan. The Impact of Sequencing Human Genome on Genomic Food & Medicine. International Journal of Genetics and Genomics. 2021. 9: 6-19.
3. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, et al. Initial sequencing and analysis of the human genome. Nature. 2001. 409: 660-921.
4. Collins FS, Lander ES, Rogers J, Waterson RH. Finishing the euchromatic sequence of the human genome. Nature. 2004. 431: 931-945.
5. Kerstin Lindblad-Toh, Claire M Wade, Tarjei S Mikkelsen, Elinor K Karlsson, David B Jaffe, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature. 2005. 438: 803-810.
6. Jay Shendure, Shankar Balasubramanian, George M Church, Walter Gilbert, Jane Rogers, et al. DNA sequencing at 40: past, present and future. Nature 550: 345-353. Nature. 2017. 550: 345-353.
7. Ross WCJ. Chlorambucil. Journal of the American Chemical Society. 2015. 136: 5860-5863.
8. Ross WCJ. The Chemistry of Cytotoxic Alkylating Agents. Advances in Cancer Research. 1953. 1: 397-449.
9. Ross WCJ. Biological Alkylating Agents. Butterworth, London. 1962.
10. Ross WCJ. 43. Aryl-2-halogenoalkylamines. Part I. Journal of Chemical Society. 1949. 183.
11. Ross WCJ. 464. The reactions of certain epoxides in aqueous solutions. J Chem Soc. 1950. 2257.
12. Ross WCJ, Mitchley BCV. Ann. Rep. Brit. Empire Cancer Campn. 1964. 42: 70.
13. Thierry Facon, Jean Yves Mary, Cyrille Hulin, Lotfi Benboubker, Michel Attal, et al. Melphalan and prednisone plus thalidomide versus melphalan and prednisone alone or reduced-intensity autologous stem cell transplantation in elderly patients with multiple myeloma (IFM 99-06): a randomised trial. Lancet. 2007. 370: 1209-1218.

14. Cobb LM, Connors TA, Elson LA, Khan AH, Mitchley BCV, et al. 2,4-Dinitro-5-Ethyleneiminobenzamide (CB 1954): A Potent and Selective Inhibitor of the Growth of the Walker Carcinoma 256. Biochemical Pharmacology. 1969. 18: 1519-1527.
15. Khan AH, Ross WCJ. Tumour-Growth Inhibitory Nitrophenylaziridines and related compounds: Structure-Activity Relationships. Part I. Chem-Biol Interactions. 1969. 1: 27-47.
16. Khan AH, Ross WCJ. Tumour-Growth Inhibitory Nitrophenylaziridines and related compounds: Structure-Activity Relationships. Part II. Chem-Biol Interactions. 1971. 4: 11-22.
17. Hameed Khan A, John Driscoll. Potential Central Nervous System Antitumor Agents: Aziridinylbenzoquinones. Part I. Journal of Medicinal Chemistry. 1976. 19: 313-317.
18. Chou Ed, Hameed Khan A, John Driscoll. Potential Central Nervous System Antitumor Agents: Aziridinylbenzoquinones. Part II. Journal of Medicinal Chemistry. 1976. 19: 1302.
19. Hameed Khan A. 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. 2021. 5: 1-9.
20. Hameed Khan A. 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. 2021. 5: 1-9.
21. Carl Djerassi, Rosenkranz G, Romo J, Kaufmann St, Pataki J. Steroids VII.1 Contribution to the Bromination of 4-3-Ketosteroids and a New Partial Synthesis of the Natural Estrogens. 1950. 72: 4534-4540.
22. Hameed AK. The Impact of Sequencing Human Genome on Drug Design to Treat Oral Cancer: Published in the Intech Open. 2020.
23. Ala-Eddin Al Moustafa. A chapter was Published in the book entitled, Development of Oral Cancer - Risk Factors and Prevention Strategies. Springer & edited. 2018.
24. Hameed Khan A. 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. 2021. 5: 1-9.
25. Leon V Berhardt. 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. Advances in Medicine and Biology, NOVA Medicine and Health. 2021. 180: 1-63.
26. Abdul Hameed Khan. The Rational Drug Design to Treat Cancers: Drug Design - Novel Advances in the Omics Field and Applications. London. 2021. 117.
27. Hameed Khan. The Impact of Sequencing Human Genome on Genomic Food & Medicine. International Journal of Genetics and Genomics. 2021. 9: 6-19.
28. Hameed Khan. The Impact of Sequencing Genomes on The Human Longevity Project. J Med - Clin Res & Rev. 2021. 5: 1-12.
29. Hameed AK. 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. 2021. 5: 1-9.
30. Khan H. The Impact of Sequencing Genomes on The Human Longevity Project. J Med Clin Res & Rev. 2021. 5: 1-12.
31. Hameed Khan. The Impact of Sequencing Human Genome on Genomic Food & Medicine. International Journal of Genetics and Genomics. 2021. 9: 6-19.
32. Hameed Khan. 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. Advances in Medicine and Biology. 2021. 180.
33. Arli Aditya Parikesit. The Rational Drug Design to Treat Cancers. Drug Design - Novel Advances in the Omics Field and Applications. 2021. 95-115.
34. Abbreviated Key Title: EAS J Biotechnol Genet ISSN: 2663-189X (Print) & ISSN: 2663-7286 (Online) Published by East African Scholars Publisher, Kenya.
35. Hameed Khan. Genomic Medicine: Using Genetic Make-up of the Human Genome, AZQ was Designed to Treat Glioblastoma, the Brain Tumor. Crimson Publishers. 2021. 6.
36. Hameed Khan. The Impact of Sequencing Human Genome on Cancer Chemotherapy. J Can Res Rev Rep. 2021 3: 1-10.
37. A Hameed Khan. The Impact of Sequencing Genomes on the Understanding of the Origin of Life on Earth. Biomed J Sci & Tech Res. 2021. 40.
38. Hameed Khan A. The Impact of Sequencing Human Genome on the Genetically Engineered Life. J. Cancer Research and Cellular Therapeutics. 2022. 6: 1-16.
39. Hameed Khan A. Sequencing the Human Genome to Study the correlation between Genes and Violence, Horizon publishers. 2022.
40. Hameed Khan A. The Impact of Sequencing Human Genome on the 2nd Genesis on Earth, Journal of Current Trends in Agriculture, Environment and Sustainability, Katalyst publisher. 2022.
41. Hameed Khan A. Assessment of Human Genome Sequencing on the New-World Order. Research Aspects in Biological Science. 2022. 4: 84-133.
42. Hameed Khan A. The Impact of Sequencing Human Genome on Novel Drug Delivery Method to Treat Cancers. International Journal of Genetics and Genomics. 2022. 10: 64-78.
43. Hameed Khan A. 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. 2022. 5: 1-21.
44. Hameed Khan A. The Impact of Sequencing Human Genome on Idiopathic Diseases 2. Biomedical Sciences. 2023.5: OAJBS.ID.000554.
45. Hameed Khan A. The Impact of Sequencing Human Genome on Darwinian Evolution. J Can Ther Res. 2023. 3: 1-26.
46. Hameed Khan A. The Impact of Sequencing Human Genome on Treating Mental Disorders Including Brain Cancer. Sci Set J Cancer Res. 2023. 2: 01-17.