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The Impact of Sequencing Tumor Suppressor p53 Gene, "The Guardian of the Human Genome" on Cancer Chemotherapy

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Abstract

The purpose of this abstract is to study the Normal Function of p53 gene which codes for making a protein called tumor protein p53 (or p53). This protein acts as a tumor suppressor, which means that it regulates cell division by keeping cells from rapidly growing and dividing (proliferating) too fast or in an uncontrolled way. In normal cells, the p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: (a) growth arrest, (b) DNA repair and (c) apoptosis (cell death). The growth arrest stops the progression of cell cycle, preventing replication of damaged DNA. The p53 protein regulates the transcription of many different genes in response to a wide variety of stress signals. p53 is the most frequently mutated gene in cancers. It is particularly mutated in some of the most aggressive cancers such as small cell lung cancer, squamous cell lung cancer, and triple-negative breast cancer. The loss of the function of p53 is a major driver of cancer development mainly because, in the absence of p53 the "Guardian of the Human Genome," cells are no longer adequately protected from mutations and genomic aberrations resulting in cancers. The couple with the family history of cancers, can still have a healthy baby if conceived by in vitro fertilization. By comparing with mutated p53 sequence with the sequence data from the 1000-genome project, we can easily identify with precision and accuracy. the specific mutated nucleotide responsible for causing the disease. Once the mutation on the nucleotide in a gene is identified, we can design drugs to shut off that gene. The long-term purpose of this study is to conquer all old age diseases including cancers, Alzheimer and cardiovascular diseases to prolong human life for deep space travel in search of new homes for humanity in the nearest exo-planet. The ultimate aim is to protect, preserve and spread human intelligence in every corner of the Universe.

Keywords: p53 Gene Disorder, DNA Repair, Apoptosis, Oxidative Phosphorylation, Glioblastoma. BBB, Aziridine, Carbamate, AZQ.

I. A Note to my Readers

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

II. Special Notes

I am describing below the use of highly toxic lethal chemical weapons (Nitrogen Mustard) which was used during WWI and its more toxic analogs developed as more toxic weapons during WWII. I described the use of Nitrogen Mustard as anti-cancer agents in a semi-autographical way to accept the responsibility of its use. When we publish research papers, we share the glory with colleagues and use the pronoun "We" but only when we share the glory not the misery. In this article by adding the names of my coworkers, the animal handers, I will share only misery. The Safety Committee is interested to know who generated the highly lethal Chemical Waste, How much was it generated and how was it disposed. I accept the responsibility. The article below sounds semi-autobiographical, it is, because I am alone responsible for making these compounds of Nitrogen Mustard, Aziridines and Carbamate. To get a five-gram sample for animal screening, I must start with 80 grams of initial chemicals for a four-step synthesis. To avoid generating too much toxic chemical waste, instead of using one experiment with 80 grams, I conducted 80 experiments with one gram sample, iso-

lating one crystal of the final product at a time. The tiny amount of waste generated at each experiment was burned and buried at a safe place according to safety committee rules.

III. Ancient References that can be Googled on your Cell Phone are Removed

Historical Background

To understand the origin and function of p53 gene, and its mutations which produces cancers, we have to explore the origin and formation of protein, DNA and life itself. Since the dawn of human civilization, we have asked ourselves simple questions like Who are we? Where have we all come from? What was it that make us this way? When did the Universe began? Why is it expanding at an accelerating? How is it likely to end? Are we alone in the entire Universe or are there other creatures in the deep dark who may or may not look like us? To move science forward, we provide the current knowledge about our origin and the origin of our Universe. Only humans in the entire Universe talk about the Universe. How and when did the Universe began? How and when did the subatomic particles interact to become molecules (Physics)? When how did molecules interact to become matter (Chemistry)? How and when did matter organized to become alive (Biology)? When and how did the first living unicellular creature become the multicellular? When and how did microbes become mosquitos and from mosquitos to mouse to monkey to men? When the first primitive man walked out of Africa three and a half million years ago, he saw for the first-time other living creatures who are so different from himself. He observed every creature is so different from one another. Since each is specifically designed, there must be a designer, a supreme being, a God. The mightiest power who resides in the sky above. Only God knows answers to all his questions. His belief was confirmed when he saw God's anger who unleased thunder and lightning which struck at a dry grass land and saw for the first time how brush fire become a forest fire. Only God knows answers to all his questions satisfied him. Questions like who are we? Where have we all come from? What was it that made this way? How the Universe began? Why is Universe expanding at an accelerating speed? How is it likely to end? Are we alone in the entire Universe or are there other creatures who may or may not look like us? He saw the exploding volcanos burning down his village to ashes; he saw the mighty water wave (tsunami) washed down his village. God knows all. His belief was spread in his village like a wildfire. To please God, he offered his most precious possession that is human life. To sacrifice a human being to please God is considered the ultimate gift to God.

The only man who challenged this belief was Darwin. In 1859, Charles Darwin published his book the Origin of Species. Darwin challenged God knows best answer to Man knows better. His answers were based on the evidence. The evolution of life from microbes to men is extremely slow process. Three and a half billion years of biological evolution brought us here from a simpler microbe to complex men. The evidence was everywhere. Entire evolutionary history is trapped in the layers of rocks as a fossil record. If we examine some of the tallest mountains on the face of the Earth, they were at some times were at the bottom of the ocean. If you break a piece of rock at the top of the mountain, you find it is loaded with microscopic shells. Using the radioactive carbon dating, you can determine the exact age of the fossil in billions of years. No human bones were found anywhere in the world except in Africa. The first complete human fossil was found in three and a half million-year-old rock found the Hader Valley in Ethiopia. These were the bones of an eighteen-year-old Black woman, we named her Lucy. We have all descended from her. She was the mother of us all. From the study of the fossil records, we conclude the following:

Evolution is any change across successive generation in the heritable characteristic of biological population. Evolutionary processes give rise to diversity in every level of biological organization including species, individual organism, and molecule such as DNA and protein. Life on Earth originated and then evolved from a Universal Common Ancestor approximately 3.7 billion years ago.

Repeated Speciation and the divergence of life can be inferred from shared sets of biochemical and morphological traits by shared DNA sequences. These homologous traits, and sequences are more similar among species that share a more recent common ancestor and can be used to reconstruct evolutionary history using both existing species and fossil record. Existing pattern of biodiversity have been shaped both by speciation and by extinction.

Charles Darwin was the first person to formulate a scientific argument for the theory of evolution by means of natural selection which is a process inferred by three facts about population: (a) More offspring are produced than can possibly survive. (b) Traits vary among individuals living to differential rates of survival and reproduction and (c) Traits differences are heritable. Thus, when several populations die, they are replaced by the progeny of parents that were better adapted to survive and reproduce in the environment in which natural selection took place. This process creates and preserves traits that are seemingly fitted for the functional role they perform. Natural Selection is the only known cause of adaptation, but not the only known cause of evolution other non-adaptive causes of evolution include mutation and genetic drift. Without any genetic or experimental evidence, more than 150 years ago, Darwin correctly predicted that a relationship exists between us and with all living creatures on Earth.

When Darwin said that we are all evolved from lowly creatures, he was right. We are not created separately in six days but are part of lowly creatures and are evolved over billions of years through a slow process of evolution and natural selection. Life evolved and nature selected. For example, the microbial life found in the boiling waters near volcanoes, cannot survive in the freezing waters of arctic oceans. Visa versa is also true. Natural selection has generated mutation which helps survive these microbial life forms in the extreme environment. It is hard for the modern-day scientists to believe that fully developed man, mouse, and monkey spontaneously appeared on Earth with all living creatures instantly by the divine order. There is no evidence for this hypothesis. Paleontologists (who study the history of fossils trapped in the layers of ancient rocks) showed that the geological evidence trapped in the layers of rock showed that it has taken ions of slow evolution and adaptation from simpler to more complex species by a slow process of evolution and natural selection. If we examine the layers of rocks of the Pre-Cambrian Age, about 530 million years old rocks, and compare them to

the present-day rocks, we find a gradual change, oldest rocks contain the simplest forms of fossil with simplest bone structure as we examine the younger and younger rocks, we find in the fossils of animals more complex bone structures showing the evidence of evolution and the natural selection of the fossils and their modification of the structure in the existing environment. No human fossils were found in the ancient rocks. Our fossils were discovered in upper most layer of about three million years old rocks.

From the study of geological records, it is certain that neither Adam nor Eve nor any other life form could have burst spontaneously on the planet in a form identical to the ancestors of today. Darwin's universe did not operate by appearance of a sudden Design, but by a slow process of evolution and followed by the natural selection based on the environmental condition existing at that time. Darwin theory proposed that evolution and natural selection is self-regulating, self-organizing and self-evolving and it requires no divine intervention. To make science and religion compatible, some people wonder if Natural Selection be a part of God's design and plan. With the rapid growth of science and technology, the idea becomes more and more critical. Darwin's evolution and natural selection is extremely slow process, and it takes eons. Mother Nature put building blocks of life together over millennia. Evolution of our Universe itself is an extremely slow process.

According to the Science of Cosmology, the Universe was a single mass of energy. May be God said, let there be light and there was light. About 13.72 billion years ago, the Universe exploded with a Titanic Force called the Big Bang. Its content spread in every direction. The Universe began to cool, gravitational forces attracted, and the material began to condense forming island of galaxies. The galaxy in which we live is called the Milky Way Galaxy. Further cooling resulted in the formation of Star Systems like our Solar System. The revolving burning material condensed around our Sun forming planets. Planets such as Mercury and Venus are too close to Sun are too hot to support life. Planets too far from Sun such as Mars, Jupiter, Saturn, Neptune, and Uranus are too cold to support life. Planet Earth is in the habitable Zone. It is neither too hot nor too cold. Life is evolved. The early hot Earth was bombarded with comets which brought water to Earth surface forming oceans. Seventy percent of Earth surface is covered with Water. Planet Earth is a Water World. In Summer, Water evaporates and in winter it condensed. Thunders and lightning storms cooled the planets even further. A million-lightnings strike Earth each day. At some remote corner of the Earth, lightning struck at a cloud of gases consisting of Ammonia, Carbon dioxide, Water near a Phosphate rocks forming the first information molecule called nucleotides.

Soon after the Earth was formed about four and a half billion years ago, the surface of the Earth was like visions of hell filled with the oceans of liquid rock, boiling sulfur, and impact craters everywhere. Volcanoes blast off all over the place, and the rain of rocks and asteroids from space never ends. Nitrogen gas released from the volcanic activity filled the atmosphere. Within a billion year, the heavy bombardment slowed down, and the surface begins to cool. The heavy bombardment of comets which brought water to Earth. The hot Earth begam to cool. Every drop of water on Earth was brought by comets. As the climate change over a billion year, there appeared the first sunshine, the first snow melts, and the first rainfall. As soon as the clouds subside, the temperature dropped to zero and the water sets an ice age.

In Darwin's world, there is no life on early Earth. Our Solar System was formed over 4.6 billion years ago. Newly formed Earth was lifeless and inhospitable place for life to survive. Every drop of water was brought by Icy comets. In the absence of sunlight, the water froze forming an Icy Age. Over eons, as the Earth warm, the Ice Age begins to disappear. There was a first Sun Rise on Earth, the first Rainfall and the first Storm on Early Earth conditions suitable for starting life on Earth surface. Life was not brought to Earth from Heaven, but was created on Earth. One wonders how microscopic life originated on early Earth. As I said above, a million-lightning strikes Earth each day. At some remote corner of the Earth on a cloud of gases such as ammonia, carbon dioxide, methane, and phosphate rock a single lightning struck creating a macromolecule RNA (Ribonucleic Acid) which is made of the same life forming four nucleotide bases. They are Adenine (A), Thiamine (T), Guanine (G) and Cytosine (C). In RNA, Thymine is converted to a more active Uracil (U). Out of four nucleotides, three code for an amino acid called Codon. Several hundred codons join to form a gene which codes for a protein. RNA is a unique molecule which can store information like DNA and catalyze reaction like protein. It is the very first molecule which initiates the early life on Earth. These molecules accumulated over eons giving rise to a more stable molecule, the Deoxyribose Nucleic Acid (DNA), amino acid and proteins the basic building blocks of life. The single cell life forms are established and within a billion years, the surface of Earth was teeming with all kinds of microbial life and one such life form was blue-green algae which conducts photosynthesis that is it absorbs Carbon dioxide and pumped Oxygen in the atmosphere. Proof of the evolutionary changes came from Wilcock's collection of pre-Cambrian fossils such as Trilobites found in Canada showed the diversity of fossils on early Earth. Early scientists wondered how these complex life forms got on Earth.

Every drop of water was brought to Earth by comets. Because of the lack of sunlight, liquid water on Earth was frozen to Ice. Over eons, Planet Earth was gently warm and slowly came out of Ice age. The climate of the late Precambrian time, the Pro-Triazoic Eon (2.5 billion years ago to 543 million years ago) was typically cold with glaciations spreading over much of the Earth. At this time, the continents were combined into a single supercontinent. A supercontinent is a landmass made up of most or all of Earth's land. By this definition, the landmass formed by present-day Africa and Eurasia could be considered a supercontinent. The most recent supercontinent to incorporate all of Earth's major—and perhaps best-known—landmasses was Pangea. The first single-celled living creatures appeared within a billion year were anaerobic and thrive in the Nitrogen atmosphere. Most of the life that existed during the Precambrian Time span were prokaryotic single-celled organisms.

Using modern molecular biology technique and comparing nucleic acid and amino acid sequences across living species, the techniques are enabling the identification of genetic components and patterns stingily conserved by evolution, from those

in which times of evolutionary branching of the tree of life can be inferred. Where did they all come from? It is easier for a religious person to understand. He would say God put them here. It was Louis Pasteur who showed that even smallest microorganisms arise from parent microorganisms resembling themselves. We wondered how the first organism appeared on Early Earth.

RNA World: The Beginning of Early Life

Millions of nucleotides joined to form a self-replicating complex molecule called RNA molecule (Ribonucleic Acid), the first information molecule for creating life. Our early Earth was filled with Nitrogen gas coming from millions of volcanos on hot Earth. Anaerobic life thrives on Nitrogen filled RNA World. Anaerobic life can store information like DNA and catalyzed reactions like proteins. According to Darwin, life evolved, and nature selected. Millions of nucleotides are created on the early Earth. When 120 to 90 thousand nucleotide base pairs (in RNA world it is A linked to U and G linked to C) joined together in an organism to form a new organelle called Chloroplast. It has a unique ability to conduct Photosynthesis that is, it can absorb atmospheric Carbon dioxide in the presence of sunlight convert to its food Carbohydrate and release Oxygen as a by-product. Oxygen gas is very toxic to the anaerobic life-forms. As more and more Oxygen is released, more and more anaerobic life-forms died. With the arrival of Oxygen by plants, RNA world ended, and more complex life-giving molecules were evolved such as DNA (Deoxy Ribonucleic Acid). The new molecule is more stable which stores information called DNA (in which A is linked to T and G is linked to C giving DNA molecule). Three out of four nucleotide code for an amino acid called Codon. The four-letter text gives 64 three letter codons. Several codons code for a protein. Proteins which carry out body function. Over eons, other new molecules appeared such as Carbohydrates to provide energy, and Hormones to support life. These are all scientific facts. Now we know the answers to these questions where they all come from not from the Heaven but were formed on Earth. This concept starts a New World Order. All life originated on Earth from the complex interactions of DNA.

DNA World: The Beginning of Established Life

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

Today, we have read (mapped and sequenced the genomes of dozens of living creatures) and identify not only the number of genes on a chromosome which occupy less than 2% of the chromosome, but also the total number of nucleotide bases and their order in which they are arranged in a species). They are all written in the same four nucleotide bases that is A-T and G-C. The traits, we inherit from our parents are written in the same four nucleotide bases. The language of life shows that a kin relationship exists among all living creatures.as Darwin predicted. If you sequence and compare the genomes of two people, you find that our book of life is 99.9% the same and if you compare our genome with our closest relations, the Chimp, in the animal world, you find that our genome is 98.9% of the sequence of the genome the same as Chimp. Just 1.1% difference gives us intelligence and conscientiousness and makes us aware of our surroundings. Minutes difference between our genomes makes all the difference, we are free, and they are in the cages. If you line up the Sequence of human genome with of the genomes of many other species, you find that chromosome #20 in human is same as the chromosome #2 in mouse. The chromosome # 4 in human is aligned with chromosome # 5 of mice. If you aligned the sequence of human genome with fish, fly or worm genomes, you find a large section of human chromosome matches with the fish, fly or with the worm genome letter by letter. These observations confirm that a kin relationship exists among all living creatures.

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

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

Essential components of life are RNA, DNA, Proteins, Carbohydrates, Lipids, and Hormones. We always wonder how these non-living chemicals could get together to create living creatures. When did Chemistry became Biology? When did life evolve? Where was it evolved? And how life was evolved? Evolution of Life on Earth is not a miracle. Life could have been evolved on Earth's surface such as on the oldest rocks found in Australia or it could have been evolved at the bottom of the Ocean floor where Black Smokers are formed with Lava emerging from under sea volcanoes reacted with surrounding Hydrogen Sulfide gas which provides energy for life forms such as tubeworms and crabs that thrive on the Ocean floor. Life also could have been

evolved underground. Soil sample brought by miners from the gold mines in South Africa two miles deep underground contained micro worms. Such life form could be cultivated on a Petri dish containing Agar mixed with nutrients. Early life could have been unicellular life forms. Could life have been brought on Earth by meteorites. Early Earth has no Water. Billions of Comets brought Water on Earth. Would it be possible that some of those Icy comets contained life giving essential components? Life could also have been evolved on the surface of Earth. The polymerization of Formaldehyde in the atmosphere could produce Carbohydrates another essential component of life. The presence of Acetonitrile, Carbon dioxide, Water in the presence of Ultraviolet light could produce the nucleotides the building blocks of life such as Adenine (A), Thiamine (T), Guanine (G) and Cytosine (C) forming a binary code leading to RNA which start replicating itself creating the first living anaerobic creature. Since no human was present to witness the formation and evolution of first life on Earth, we rely on its presence from the early fossils found in the layers of ancient rocks.

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

The Geologic Clock

Let me scan the origin of life on planet Earth from the very beginning to the present day. The slow evolutionary changes can be explained from three and a half billion years to the present day. If we were to examine the fossil record based on the Geologic Time Scale, we can divide this time into three great eras. First, The Paleozoic Era which starts from the very beginning of the Pre-Cambrian Era starting from the 100 million to 400 million years ago. Second, The Mesozoic Era beginning from 230 million years to 70 million years ago. Third, The Cenozoic Era beginning from the 63 million years to the present day.

As more and more chloroplast genomes accumulate, a primitive life form the Blue Green Algae appeared which act as primitive plant life. Plants first appeared on the Planet Earth about 400 million years ago. With the appearance of plants, Oxygen molecules accumulate in abundance. The photosynthetic apparatus, the chloroplast, in plants started absorbing Carbon dioxide and started releasing Oxygen in the atmosphere. As forests thrive in the aerobic atmosphere, the evolution in many plants played significant role in converting the Carbon dioxide to Oxygen. Of all the plants, Maize is the winner. The fields of plants maize can efficiently assimilate and convert CO2 to carbon products such as Carbohydrates during photosynthesis releasing more Oxygen. As the Sun rays strike the forests on Earth surface, the Chloroplasts in the trees convert the Physical sunlight energy into Chemical energy by photosynthesis. Most of the Earth is powered by photosynthesis. It was plants that introduced Oxygen in Earth's atmosphere.

During the Pre-Cambrian Era, primitive life forms appeared. No fossil was found of the soft tissues body creatures appeared except some of the impressions of their fossils are found on the ancient rocks. During the end of the Pre-Cambrian era, some hard-shell fossils, made of Calcium Carbonate, like Trilobites are found. During Cambrian Era, about 100 million years ago, primitive life forms appeared such as Algae, Orthopods; much later sponges, worms and mollusks appeared.

Earliest life forms with shell are preserved as fossils. A treasure of fossils was discovered in British Columbia, Canada, called the Burgess Shale, found in the Canadian Rockies of Canada. These are the fossils left behind by the creatures of Middle Cambrian Era. Part of this treasure is on display at the Smithsonian Museum in Washington, D.C., Among those fossils were sea cucumber, worms, and Trilobites. During the Ordovician Period, from 425 to 500 million years ago, bony life forms appeared which include Tetracorals, echinoids, asteroids appeared for the first time on the primitive Earth. During the Silurian Period, (from 425 to 405 million years ago), brought the most dramatic changes in the Earth's atmosphere. Tall plants appeared for the first time. Up to this time, Earth's atmosphere was full of Nitrogen gas released by the cooling of the hot Nitrate containing volcanic Rocks. Earth's atmosphere also contained the Carbon dioxide contributed by the Volcanic eruption. It was the appearance of Chloroplast that changed the direction of life on Earth. For the following 60 million years, at the beginning of the Devonian period, the cooled part of the planet Earth, was carpeted by the early plant life called the Blue Green Algae. Its job is to absorb Carbon dioxide and release Oxygen. Over millions of years, enormous amount of Oxygen was released in the atmosphere.

By the end of the Silurian Period, the composition of the Earth's atmosphere was changed from pure Nitrogen gas to 80% Nitrogen and 20% Oxygen gas. The gas Oxygen is extremely reactive, it reacted with the Oceans Iron forming the Iron oxide, the rust. Billions of tons of Iron Oxide deposited on the Ocean floor. Oxygen is toxic to the Anerobic life forms. Those creatures who survive in the presence of Oxygen thrived while Anerobic life forms died. More complex life forms appeared rapidly.
Darwin's remarkable foresight about the evolution of life on Earth can be confirmed by modern methods using DNA sequencers. By using next generation Nanopore Sequencer, we can read the number and order of nucleotides at each evolutionary stage. We can demonstrate how at each step of evolution some old life nucleotides are deleted and others are inserted to survive in a changed environment and how new species are evolved.

The formation of early life on Earth was demonstrated by making the building blocks of life the amino acids which carry all body functions, was synthesized in the Labs. In 1953 Stanley Miller, the student of the Nobel Laureate, Harold Urey, at the Chicago University conducted an experiment in the Lab to create life's essential components the amino acids. He created primitive Earth like conditions in a flask in the Lab. He took two flasks connected with a condenser. One flask contained water vapors and the other filled with gases found on the primitive Earth such as Methane, Carbon dioxide and Ammonia. To mimic thunder and lightning, a source of energy, on Earth, he sparked electric current in the flask. The high energy electric spark, split the stable molecules of Nitrogen, Oxygen, and Carbon, produc-

ing extremely reactive ions which reacted with one another recombing to produce a more stable new molecule. Within a week, the clear solution in the flask became pink and dark. The analysis of the colored material showed the formation of Amino Acids, the essential building blocks of life which perform all body functions. In similar experiments, Francis Crick and Lesli Orgel, attempted to synthesize Nucleotides the replicating molecules which carry information to make life. Using Formaldehyde, the other essential components of life such as sugars and hormones were synthesized.

In 1944, Irvin Schrodinger wrote a book called "What is Life?" in which he described that life is governed by a secret code he called "The Genetic Code". Long before the formation of the building blocks of life to confirm the arrival of life on Earth, it was Schrodinger who predicted the arrangements of the nucleotides which forms a code carrying the secrets of life. He was the first person to predict it as, the genetic code.

In 1949, it was Irwin Chargaff who discovered that the essence of life is information and the information is located on the long string of DNA consisting of four nucleotide and these four nucleotides are Adenine (A), Thiamine (T), Guanine (G) and Cytosine (C) and they exist in one-to-one ratio. It comes to be known as Chargaff's rule. Chargaff's rules state that DNA from any cell of all organisms should have a 1:1 ratio (base Pair Rule) of pyrimidine and purine bases and, more specifically, that the amount of guanine is equal to cytosine and the amount of adenine is equal to thymine

In 1953, the genetic code was broken by Crick & Watson [1]. They discovered that the four nucleotides discovered by Chargaff when join, they form a helical structure. They stack on top of each other. They also twist around like a spiral stair case. The structure shows how life works. DNA unwinds to form two more helix. One of them is original copy. Replication is the copying mechanism in all life forms. The helical structure of DNA carries the script of life. DNA is a molecule that controls life. This script is passed on from cell to cell, from generation to generation and from species to species in all life forms from a tiny blade of grass to mighty elephants including man, mouse, monkey and microbes. DNA is not just the self-replicating molecule; it is the essence of life carrying information from generations to generations. These information molecules are to make all life forms. For scientists, life is no longer a mystery. DNA unzipped the double helix and copy off one of the two stands. The other copy is released for another strand of the process. During replication, DNA molecule pick up the nucleotides from the chemical soup forming the double helical structure of DNA. The double helical structure of DNA was confirmed by X-ray diffraction, (by Wilkins & Franklin) provides a mechanism of replication in which A is always linked to T and G is always linked to C. The product of the gene are proteins which makes man to mouse to all life.

I have immense admiration for those four persons who determined the double helical structure of DNA without the formal training of Organic Chemistry. The only chemist's name appeared in those days was Professor Irwin Chargaff who determined the presence of four nucleotides bases, Adenine, Thiamine, Guanine and Cytosine in the DNA in the ratio of 1:1. His important contribution was never recognized and he died a biter man at 96.

It was the darkest days for Organic Chemists During WWI, chemical weapons were introduced. Weapons such as Nitrogen Mustard and Nerve gases which killed millions of soldiers and civilians in their trenches and homes. It was Chemistry's darkest hour.

Sun began to shine on chemistry profession almost twenty years after the WWI, When Ross, a professor of Chemistry in the University of London, England, observed that soldiers exposed to Nitrogen Mustard during WWI, showed sharp decline of WBC. He realized that children with Leukemia showed a sharp increase of WBC. He wondered if minimum amount of Nitrogen Mustard could be used to control Leukemia in children. It is indeed found to be true. Over Decades, Ross made hundreds of analogs of Nitrogen Mustard. Among the most useful are Chlorambucil, Melphalan and Mero Phan for treating different kind of cancers. Because of presence of Nitrogen Mustard moiety in the drug. Ross was never honored for his work. Neither his students who worked on making Nitrogen Mustard analogs because Nitrogen Mustard analogs cross-link both strands of DNA killing the cells.

I served Professor Ross for almost ten years, first as his student, then as his postdoctoral fellow then as his special assistant. During those years, I was given a different path to follow, instead of cross-linking both strands, I was to bind to a single strand of DNA using Aziridine (half the Nitrogen Mustard). Over the years, I made more than one hundred analogs of Aziridine. When I made Aziridine, 2-4-dinitrobenzamide (CB1954), It showed antitumor activity seventy time higher than ever recorded against the solid aggressive Walker Carcino 256 in Rats. For my doctoral thesis, I made 91 analogs of CB1954. For my work, I was honored with the ICR (Institute of Cance Research) Postdoctoral Award of the Royal Cancer Hospital to continue to make more analogs of Aziridine. When I received ICR award, most of us thought the curse on Nitrogen Mustard is over until, I made an extremely toxic compound, Aziridine, 2-4 dinitrophenyl Carbamate. It was so toxic that its toxicity could not be measured. Further work on its analogs were stopped. Although further work on Aziridine Carbamate was stopped at the London University, it continued in America when I was offered the Fogarty International Fellowship to continue further work at the National Cancer Institute at NIH, Bethesda, Maryland, USA.

It was Aurther Kornberg who discovered the enzyme DNA polymerase. When added in the solution of a mixture of A-T and G-C, the nucleotides become alive they arrange themselves to form polymers. A string of A-T and G-C is called the Deoxyribonucleic Acid or DNA. When DNA polymers cut to pieces by molecular scissors (called Restriction Enzymes) and join together by an enzyme DNA ligase, Obeying the laws of Physics, Chemistry and Biology, these fragments organized themselves to become alive.

A string of nucleotides is called the DNA (Deoxyribose Nucleonic Acid). A small section of the long DNA codes for a protein called Gene. A gene is a unit of inheritance and its codes for a protein. A gene on the DNA is identified by the presence of a Start codon AUG which codes for the amino acid Methionine and at the end of the gene there are three Stop codon and they are UGG, UAG, UGA. At the end of the one of the Stop codons, the non-coding DNA continues.

Hundreds of gene are embedded on a single long strand of DNA. For example, Chromosome-17 is made of 92 million nucleotides base pairs and less than two percent of the DNA carries three letter codons which code for amino acids. Hundreds of codons join to form a gene which codes for proteins. A gene is a unit of inheritance. It is identified on a DNA by a single Start codon and one of the three Stop codons. The Start codon of a gene is AUG which codes for amino acid Methionine and the three Stop codons are UAG, UGG, UGA. Once one of the Stop codons appears, DNA extension stops. A computer program could easily identify the number of AUG codons on the long string of DNA. Damage to DNA is caused by radiations, chemical/viral infections, genetic inheritance, or DNA deletion, insertions, relocation, inversion etc. resulting in mutations causing diseases. Among the old age diseases, Cancer reigns supreme, followed by cardiovascular disease and Alzheimer. Among the deadliest is Cancers which kill more than 609,360 people in the United States last year. One of the most important gene is called p53 gene whose proteins protect the spread of cancers

On a long string of Chromosome-17 which is composed of 92 million nucleotide base pairs, we have identified 1,394 genes. One of those gene is p53, which codes for tumor suppressor protein. It prevents the spread of cancer. It is regarded as the Guardian of Human Genome. When p53 gene is damaged or mutated, either by X-radiations, chemical/environmental pollution, viral infection or genetic inheritance or viral infection, the protection is lost. The mutated p53 gene gives rise to a stable mutant protein whose accumulation is regarded as a hallmark of cancer cells. Mutations in p53 proteins not only lose their tumor suppressive activities of p53 but often introduce additional oncogenic functions that endow tumor cells with growth and survival advantages.

Genetic Engineering

Once the genes are identified on a long string of DNA, our next challenge is to cut, paste and copy a single gene. Molecular scissors (called Restriction enzymes) were discovered during war between Bacteria and Viruses. A restriction enzyme is a protein isolated from bacteria that kill viruses by cleaving DNA sequences at sequence-specific sites, producing DNA fragments with a known sequence at each end. The use of restriction enzymes is critical to certain laboratory methods, to cut and paste a piece of DNA used in recombinant DNA technology and genetic engineering. If we generate two DNA fragments with matching ends, we can join them by the enzyme DNA ligase which seal the gap between the molecules forming a single piece of DNA. Restriction enzymes and DNA ligase are often used to insert genes and other piece of DNA into plasmids during DNA cloning. Once the role of restriction enzymes was established, the two times Nobel Laureate Fred Sanger, put together a non-replicating virus, accurately that read the number and the order of the genetic letters (called sequencing) in its book of life called Genome; it has 5,386 (Kilo-base) genetic letters to be précised in this creature. Scientists have successfully put those letters together in that exact order and injected to a bacterium. The unfortunate bacteria read its code as its own and inserted in its genome. Where Phage integrates its DNA into the host cell DNA and multiplies into thousands of its own copies and kills the bacteria. Over eons, bacteria have learned to fight back the Phages by producing an enzyme called the Restriction enzymes which acts as scissors cutting the DNA into pieces. Several hundred restriction enzymes have been isolated which cut DNA at specific sites. Restriction enzymes, also called restriction endonucleases, recognize a specific sequence of nucleotides in double stranded DNA and cut the DNA at a specific location. They are indispensable to the isolation of genes and the construction of cloned DNA molecules. Restriction enzymes can be used to splice and insert segments of DNA into other segments of DNA from any other species, thereby providing a means to modify DNA and constructing new forms.

As described above, Fred Sanger created the first living organism in the Lab called Bacteriophage Phi X 174. The phi X 174 bacteriophage is a single-stranded DNA virus that infects Escherichia coli, and the first DNA-based genome to be sequenced. Creation of first life form in the test-tube started a New World Order. Creation of life on Earth is no longer a magical, mystical process sent on to Earth from Heaven in seven days is over. Instead, according to the new world order, the creation of life on Earth is extremely slow evolutionary process taking billions of years. Geological records confirm this fact. The creation of life in the Lab opens the gates to create all kinds of useful life forms to produce new food to feed over eight billion people on Earth and provide new fuel to run the engine of modern society and new medicine to treat every disease known to mankind.

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

In 1990, US Congress authorized three billion dollars to our Institute (NIH) to decipher the entire human Genome under the title, “The Human Genome Project” We found that our Genome contains six billion four hundred million nucleotides bases half comes from our father and another half comes from our mother. Less than two percent of our Genome contains genes which code for proteins. The other 98 percent of our genome contains 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 Pseudo-genes 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 diabetics around the world.

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

The Advantages of Sequencing Human Genome

The knowledge gained by sequencing human genome has summarized the past 150 years of genetic science. We have taken away the power from Mother Nature to alter billions of years of our evolutionary past. We now have all the tools we need to alter the genetic make-up of our species. Genetic Revolution has taught us that Darwinian evolution can be hastened by the rules of genetic engineering. By using the genetic tool kits, we can cut, past, copy and sequence a gene in days not in eons. The development of new tools like CRISPER-Cas 9 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 old age diseases, frequent genome sequencing will help us identify a single gene mutation that will begin to grasp more complex genetic patterns that could lead to polygenic or multigenic conditions such as Coronary Heart diseases, Cancers, and Alzheimer. Early detection will help us control their expansion. Some genes are activated at the later part of our life causing serious illnesses. If there is a family history of such diseases, frequent sequencing becomes more important for early detection.

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

The Tumor Suppressor, p53 Gene, the Gradian of Human Genome

The human p53 gene is located on chromosome 17 which is made of 92 million nucleotide base pairs carrying 1,394 genes. The P53 gene, located on the short arm of chromosome 17p13, consists of 11 exons coding for a nuclear phosphoprotein, which can bind to specific DNA sequences acting as a transcription factor. This section of the gene codes for tumor suppressor protein. Any mutations in this region whether the point mutation or other genetic defects such as DNA deletion, insertion, translocation or inversion will offer no protection and promote cancers. Genes code for protein. The p53 gene codes for p53 proteins. The p53 gene codes for tumor suppressor protein which could be used to treat cancers. Our challenge is to make large enough quantities of p53 protein to test its antitumor activity against a large number of tumor cell lines. Making large quantity of p53 protein presents the same problem as we faced when we were making large quantity of Insulin to treat diabetics either by using biological method or synthesizing the entire sequence in the Lab.

Couple with family history of cancers can still have normal children by either in vitro fertilization and implantation of healthy embryo or drug treatment after the birth of the baby. Cheaper and safer method of having a healthy baby is by in vitro fertilization After three days of conception, from the 8-cell embryo, one cell can be removed for sequencing. By comparing with Reference Sequence, if the mutations are identified in the embryo, we can discard the defected embryo and use the next healthy embryo. On the other hand, developing new drugs for treating defected fetus is time consuming and highly expensive. For example, once the gene and its site-specific mutation is identified, allele specific drugs could be designed which could be used in combination with the development of other synthetic toxic derivatives may lead to better efficacy and expanded therapeutic options for patients with altered p53 function. 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 mutated p53 cancer causing genes.

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 (Mitochondria provides unlimited energy to the host cell. In return the host cell provides free accommodation, unlimited free food and full protection) and 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 healthy 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 of mitochondria, 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 mitochondrial donation process is safe and approved the donation of an egg with healthy mitochondria. A third parents' baby was born in England and live 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.

The Mechanism of Action of p53 Gene and the Development of its Treatment

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

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

If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in ear-

ly adulthood. Cell cycle arrest and apoptosis are the most prominent outcomes of p53 activation. Many studies showed that p53 cell-cycle and apoptosis functions are important for preventing tumor development. p53 also regulates many cellular processes including metabolism, antioxidant response, and DNA repair. Activated p53 promotes cell cycle arrest to allow DNA repair and/or apoptosis to prevent the propagation of cells with serious DNA damage through the transactivation of its target genes implicated in the induction of cell cycle arrest and/or apoptosis. Upon activation, p53 induces the expression of a variety of gene products, which cause either a prolonged cell- cycle arrest in G1, thereby preventing proliferation of damaged cells, or apoptosis, thereby removing damaged cells from our body.

How to Design Drugs to Shut Off p53 Mutated Gene?

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

By comparing p53 mutated gene sequence with Reference Sequence, we can easily identify the specific mutation on p53 genome responsible for causing the cancer. In my Lab, at the Drug Development Branch of the National Cancer Institute, at NIH, we used to find dyes to color tissues. Once a specific dye is found, we used to attach highly toxic Aziridines and Carbamate moieties to attack the tumor of that tissue. We have 220 tissues in our body, we can find a color for each tissue. Enormous number of color combinations are available. For example, with four nucleotides we get 64 nucleotide combinations. The Rainbow has seven colors, can you imagine how many color-combinations. are available. During summer holidays, with a small grant to high school students, we can easily find a color for all 220 tissues. Using Dinitrophenyl dye, my work above described how to design drugs by attaching Aziridine and Carbamate to shut off a gene responsible for causing animal cancer (Walker Carcinoma-256 in Rats). and then using Quinone as a carrier for Aziridine and Carbamate how I made AZQ. By making AZQ (US Patent 4,233,215), I have demonstrated how to design drugs to shut off genes responsible for causing Glioblastoma, the brain cancer. It is the challenge for the next generation of scientists (my students) to activate the prodrug moieties derivatives of Aziridine and Carbamate to shut off mutated p53 genes to prevent cancers.

Using Nitrogen Mustard to Design Drugs to Shut Off a Mutated Gene?

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

Ross' Rationale for Using War Chemicals to Treat Cancers

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

Ross was the first person to use war chemicals successfully to treat cancer. Although such drugs are highly toxic, more cancer cell will be destroyed than the normal cells. Over decades, Ross made several hundred derivatives of Nitrogen Mustard as cross-linking agents. Some of the Nitrogen Mustards are useful for treating cancers such as Chlorambucil for treating childhood leukemia (which brought the WBC level down to 5,000/CC) and Melphalan and Myrophine for treating Pharyngeal Carcinomas. Because of the high toxicity of Nitrogen Mustard, new drugs could not be developed to treat other types of Oral or Lung Cancers [7-12].

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

Nitrogen Mustard was mercilessly used as a weapon during the WWI by both German and Italian Armies against Allied forces. Most soldiers exposed to Nitrogen Mustard were freeze to death. Their blood analysis showed a sharp decline in White Blood Cell (WBC). Since patients with the cancer of the blood called Leukemia, showed a sharp increase in WBC, Professor Ross and his group at the London University, England, wondered if minimum amount of Nitrogen Mustard could be used to control Leukemia in cancer patients. It was indeed found to be true. During the following 30 years, Ross developed hundreds of derivatives of Nitrogen Mustard to treat a variety of cancers. His most successful drugs are Chlorambucil, Melphalan and Myrophine [13]. As his graduate student, during the following ten-year period, I made for Professor Ross dozens of analogs of Nitrogen Mustards. The deadliest among them was the Phenylenediamine Mustard. We use these compounds to check the sensitivity of the Experimental Tumors in the Tumor Bank. If tumors in the Tumor Bank become resistant, we must replace resistant tumor cells with fresh more sensitive tumors for testing other compounds.

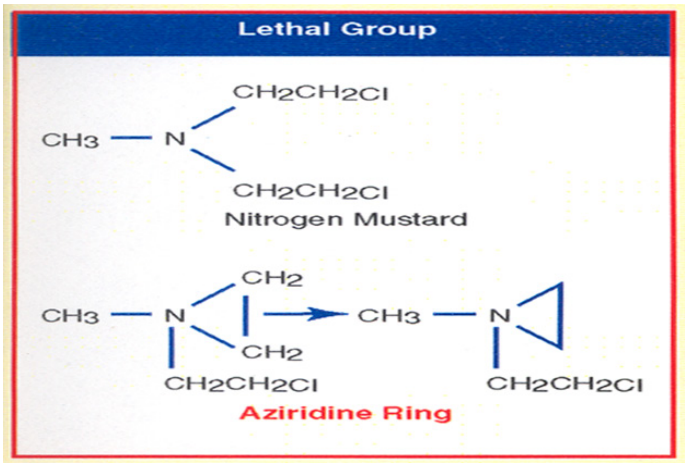
Nitrogen Mustard Shut Off a Gene by Cross-Linking Both Strands of DNA

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

Shutting Off a Gene by Binding to a Single Strand of DNA

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

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



DNA Binding Aziridine Group

This study showed that to attack a single strand of DNA, we must synthesize Aziridine in the Lab by using ethyl amino methyl sulphurated in sodium hydroxide. Pure Aziridine was distilled

off. Synthesis of Aziridine analogs will give two advantages over Nitrogen Mustard: first, instead of cross-linking, Aziridine binds to one strand of DNA, reducing its toxicity of the double stranded Nitrogen Mustard by half. Second, it gives selectivity, the Aziridine ring serves as a prodrug. Its ring opens only in the acidic medium. Once the active ingredient Aziridine was determined to attack DNA, the next question was what drug delivery method should be used to deliver Aziridine at the tumor site.

The Above Structures Are Nitrogen Mustard (2-bischloroethyl methyl amine) And Aziridine.

DNA Binding Lethal Groups

Designing Drugs to Bind to a Single Stranded DNA to Treat Animal Cancers

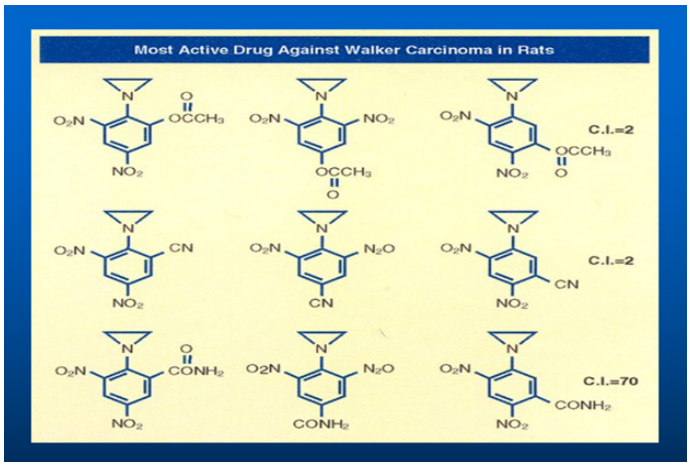
As a part of my doctoral thesis, I was assigned a different path. Instead of cross-linking both strands of DNA by Nitrogen Mustard, I am to design drugs to attack only one strand of DNA by making Aziridine analogues. We decided to use Aziridine moiety (as an intermediate of Nitrogen Mustard) that would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

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

Structure-Activity Relationship

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

An additional substituent in the Dinitrophenyl Aziridine could give three isomers, Ortho, Meta, and Para substituent. Here conformational 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 isomers of the most water-soluble **Ethyl Ester group** attached to 2,4-Dinitropehny aziridine. The compound in vivo is hydrolyzed Ethyl Ester to produce most water-soluble carboxylic group. Since it is the most water-soluble substituent, within 24 hours of injection in Rats, the entire radioactive compound was passed down from in the Rat urine and it can be extracted by Chloroform. Since the Ortho position was not available for DNA binding, it showed no biological activity, but the third compound in which Ortho position was free to bind to DNA showed some anti-tumor activity in Rats.

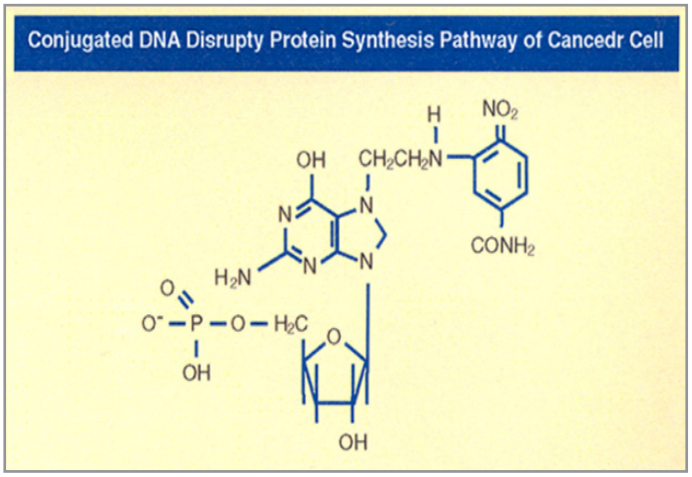
The Least Water-Soluble Substituent

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

The Moderately Soluble Amido-Substituent

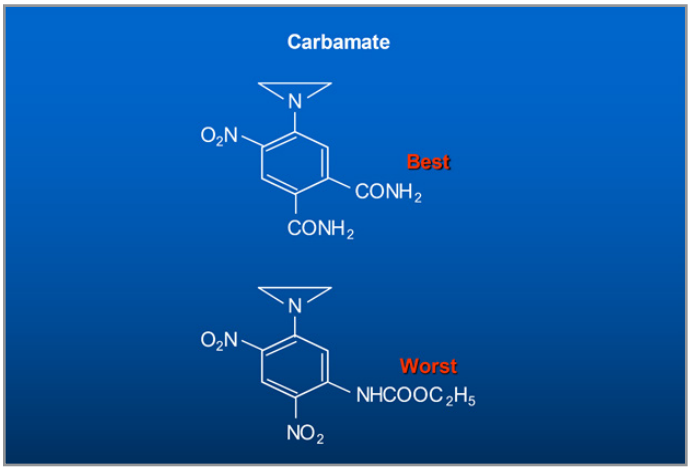
The last line of the above chart showed that the first two Amido groups were sterically hindered and did not bind to DNA and showed no biological activity, but the last compound presents the perfect drug delivery method. The entire drug was delivered from the injection site to the tumor site. The drug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

As I said above, Nitrogen Mustards are highly toxic because they have neither specificity nor selectivity. They attack all dividing cells whether they are normal or abnormal. On the other hand, the analogs of Aziridines and Carbamates serve as 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.

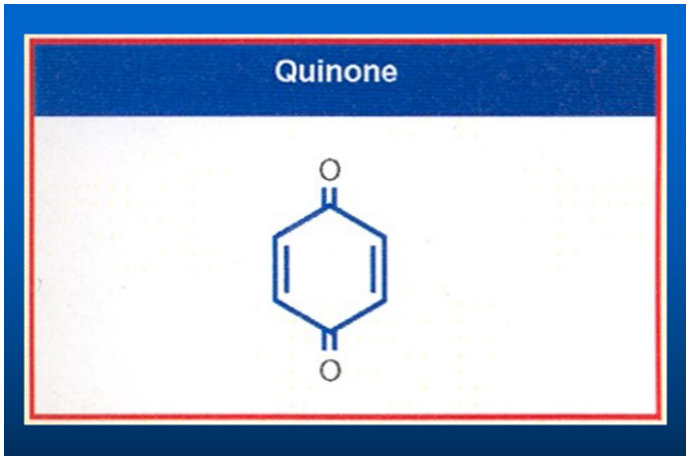
As a part of the inter-government agreement between UK and USA, all novel drugs developed in England were sent to the National Cancer Institute (NCI) in America for further screening. To translate animal work to human, I was invited to continue my work on the highly toxic Aziridine/Carbamate combination 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.

The Rational for Designing Drugs to Treat Glioblastoma, The Human Brain Cancers

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



The Structure of a Non-Toxic and Non-Addictive Quinone Used for Crossing the Blood Brain Barrier (BBB)

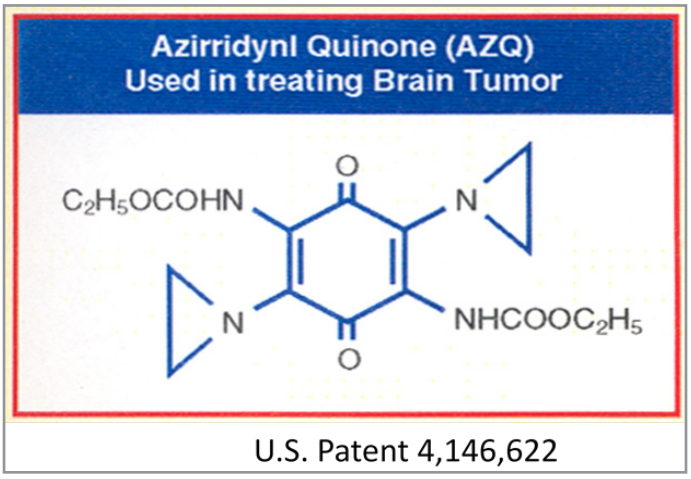
With the Quinone ring, I could introduce two Aziridine rings and two Carbamate moieties and could create havoc for Glioblastoma. Within three years, I made 45 analogs of Quinone. One of the Quinones carries two aziridines and two carbamate moieties which was highly toxic to Glioblastoma. The tumor stops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



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.

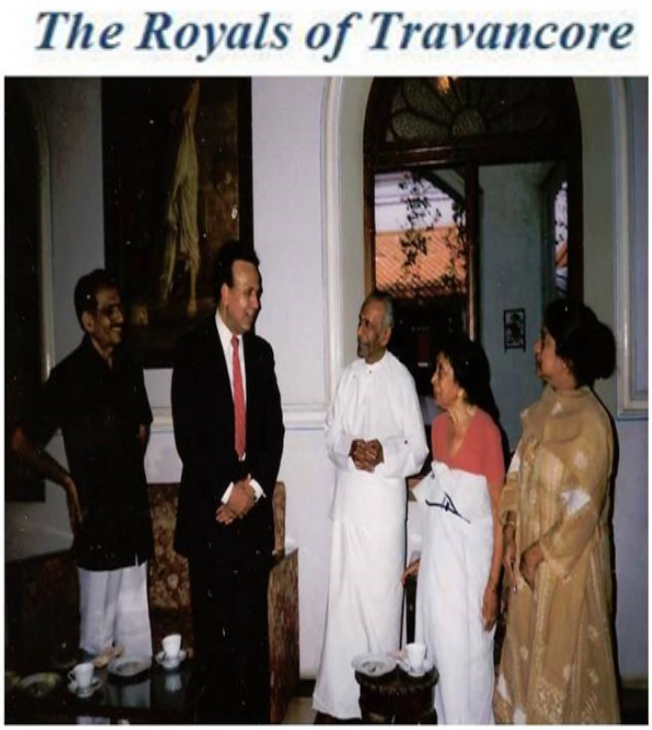
2004 NIH Scientific Achievement Award

Exhibit # 2

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

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

Exhibit # 3



Dr. 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.

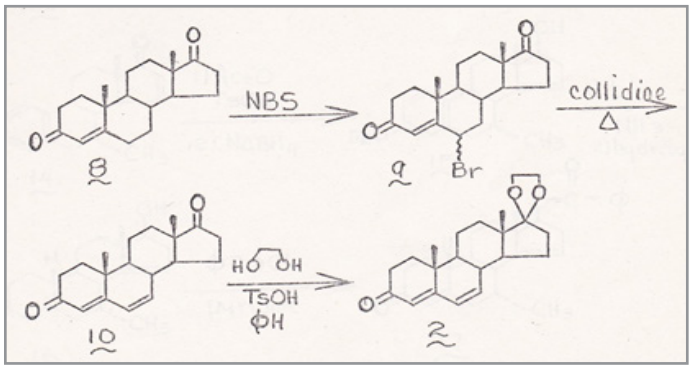
While Genome Center at NIH is supporting research on sequencing and mapping of the Genomes, my Institute NICHD was supporting research on Gene Markers associated with diseases.

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, [19, 20]. I could show how many radio-labeled nucleotides were bound to which mutations. Out of seventeen positions available for substitutions on Testosterone. There are only three positions that is 1,3 and 17 positions are available on Testosterone ring system. I could activate position 9 and 10 by reacting with Bromo-acetamide which introduce a Bromo ion on position 10 which could be dibrominated by Collidine to introduce a 9,10 double bond which I could further brominate to produce 9,10 dibromo compound. These bromo ion could be replaced by additional Aziridines or Carbamate ions. I could increase or decrease the number of Aziridine and Carbamate ions to get the maximum benefit by further brominating position 15 and 16 to introduce additional Aziridine and Carbamate moieties.



Carl Djerassi [C. Djerassi et al. J. Amer. Chem. Soc. 72. 4534 (1950)] had demonstrated that we could activate additional positions for substitutions on hormone ring system such as the position 9 and 10 by reacting with Bromo-acetamide which introduce a Bromo ion on position 10 which could be de-brominated by Collidine to introduce a 9,10 double bond which we could further brominate to produce 9,10 dibromo compound [20, 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) [22-48]. will use this method to develop drugs to treat all cancers.

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

Using genetic engineering technology, suppose we cut, paste and copy the p53 gene to scale up its p53 proteins and to supply the p53 protein to cure many forms of cancers, we may not succeed tomorrow but what about day after tomorrow. From treating cancers, we can go on to treat other two old age diseases such as cardiovascular diseases and Alzheimer. What is the result of such successes? We are most likely to have a large number of old people. In most retirement homes residents have already passed age 80. Good food and best medicines have kept them healthy. Other age prolonging studies (The discovery of TRT genes) are also underway. The tail end of the all chromosomes, we find a six- letter code (TTAGGG) called Telomers which shorten each year shortening the age. To reverse aging, an enzyme called Telomerase Reverse Transcriptase (TRT) has already been discovered which prevent aging. Another study is on its way about sequencing the genome of Centenarians to find the gene that keep them live beyond one hundred years. What is the result of these studies, the result is that most old people will live beyond one hundred years. There are more than eight billion people live on planet earth. Each year, we are adding a hundred million new mouths to feed. With this speed of increasing, the world population is most likely to reach ten billion by 2050. Can we provide, food, shelter, job to all those people. How can you prevent massive pollution, street riots, starvation, homelessness?

We need to solve these ethical problems. One group says enough is enough and the time has come to control the population, particularly the quality control of the pollution. The other group says to protect the future of humanity, we must continue to produce high quality people to protect, preserve and spread human intelligence in every corner of the Universe. Humans on Earth is trapped in the middle age dying star system. Our Sun has been burning for the past four and a half billion years. It has used up more than half of its energy. As it cools, the Sun begins to expand, swallowing the nearest two planets, Mercury and Venus as it continues to expand, its outer rim reaches the Earth, the intense heat boils off our oceans. The excessive heat burns and incarnate all life on Earth. Within four billion years, the Sun would have used up most of its energy. It will not expand any further. It will collapse on itself exploding as Supernova. The gravitational forces holding our solar system of nine planets and 140 moons will fall on itself and explode with Titanic force resulting in the destruction of our Solar System.

Humanity has come on a cross-road. One path leads to total annihilation and destruction of life on Earth, the other provides a chance to escape. We have to make a choice. One group believes that God has created Heaven and Earth and He alone has the power to save or destroy His creation including us. We live as God wishes. The other group thinks that God has given us enough knowledge, intelligence and wisdom to build the spaceships to escape Earth and travel into deep space to explore His creation and to spread human intelligence in every corner of the Universe. The first path requires nothing from you except prayers. You are on a conveyor belt to Heaven.

The other path requires enormous effort from the second group. Preliminary effort has been very successful. First to increase human age beyond one hundred years, we have discovered TRT gene. Next, we can build fleets of city-sized spacecraft for vertical takeoff like Orian Spacecraft, with new material impenetrable to heat, cold and radiations. Next to develop fusion energy to provide unlimited source of energy to propel spaceships in any direction at least with half the speed of light. Next, we must achieve to recycle all life-saving elements, including air, and water in our spaceship for long distance travel. More than five thousand exo-planets have been discovered so far, the nearest is light years away. To reach one of those exo-planets, we need to increase human age. We don't want to die on our way to settle on a new home for humanity.

To protect human life from total destruction, we need outstanding men and women who carry the spirit of the earlier greatest generation of the past century. Those outstanding men and women who demonstrated a colossal courage and outstanding energy to climb the tallest mountain, gone to the bottom of the deepest ocean, split the heart of atom and walked on the surface of Moon and returned to Earth safely. We need those kinds of men and women now. Who among you would be the vanguard of research and technology, in the greatest country in the World. We bequeath the future of this greatest country in your hands. We know you will do your best to keep America the sole remaining super power of the World, A jewel in the crown, a beacon of light and a shining city on the Hill.

The Ideas Expressed in This Article Are Mine and Do Not Represent NIH Policy

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