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Review Article

The Impact of Sequencing Human Genome on the Genetically Engineered Life

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Abstract:

This abstract describes the enormous advantages of redesigning existing microbial life in the Labs which will carry additional instructions not only to clean up our environmental pollution, but also to produce new food, new fuel, and new medicine to treat every disease known to mankind. Using the toolkit of genetic engineering developed during the completion of the Human Genome Project, we will manipulate microbial life in which we will splice essential amino acid codons in most consumable food such as Corn, Wheat and Rice Genomes to produce the most nutritious food for the bourgeoning population of the world. Similarly, new fuel could be produced by an organism called Methanococcus Jannachil which thrives near high temperature high pressure hydrothermal vents at the bottom of the sea floor by converting Carbon dioxide (a pollutant) to Methane (a fuel). To produce at the industrial scale, in plant genomes, we will splice not only the genes of herbal medicine (such as Artemisinin, Taxol, Reserpine, Belladonna etc.) to produce well-known herbal medicine, but also will insert genes to produce large scale antibiotics (such as Penicillin, Streptomycin, Neomycin, Kanamycin, Paromomycin, Apramycin, Tobramycin, Amikacin, Netilmicin, Gentamicin etc.). At every step of the transgenic genomes, we will confirm the spliced novel genes by using cheaper and faster nanopore gene sequencer. Current speed of developments guarantees humanity's future survival across the Universe long before our Sun dies

Key Words: genetic toolkit; nanopore sequencer; antibiotics; herbal medicine; transgenic

Historical background:

We broke the genetic code and unlocked the secrets of life. Now, we are ready to manipulate life not only to clean up our environment pollution, but also to produce new food, new fuel, and new medicine to treat every disease known to mankind. At dawn of new millennium, we embarked on three great revolutions in science: The Quantum revolution, the Computer Revolution, and the Genetic Revolution. These scientific revolutions deal with matter, the mind, and the life. In Quantum Revolution, we study matter. We discovered that the total matter in the Universe is made of atoms. We study a single atom. We found that the central positive nucleus revolves negatively charge electron carrying a unit amount of energy called the quantum energy. It is the nucleus that carry tremendous amount of energy. What if we break the nucleus? The nucleus is made of made of positively charged proton and neutral neutrons tightly held together by nuclear forces. What if we break the nuclear bonds by hitting with a beam of high energy neutral particles? The positively charged protons repel each other fly apart with great force hitting the neighboring nucleus breaking their nuclear bonds. This process set a chain reaction until total matter is converted into energy. With a football size pure Uranium-235; we could release enough energy to cities like Hiroshima and Nagasaki in minutes. What if we control the chain reaction by placing on its path a sheet of Boron which absorbs protons? We should be able to control the chain reaction and the energy could be converted to electricity which runs the engine of modern society. The greatest achievement we made from the Quantum Revolution is that we learn to convert matter into energy which runs the engine of modern society.

The second is the Computer Revolution. It is the revolution of mind. It brought the Information Age. Using two numbers zero and one, we wrote programs to store all the information we have generated from the dawn of human civilization to the present day. We not only store information, but also, we also learn to cut and paste, and process and move the information with the speed of light. The greatest achievement we made from the Computer Revolution is that we captured space time. We could send the information around the world in seven second.

The greatest revolution of them all is the Genetic Revolution. We solved the mystery of life. Now, we know with pretty certainty, how life could have evolved at some remote corner of the world about three and a half

billion years ago and how it crawled on evolutionary path for three billion years when it became so advanced that it could fly at will or stay still. In three billion years journey across time, it reached us. It helped us developed our language, our minds and our conscientiousness. We are the most intelligent of all living creatures; in fact, we are so intelligent that we asked ourselves simple questions such as who are we and where have we all come from and what was it that made us this way? How biological life is created, developed, and evolved on Earth? Is there a relationship exists among all living creature on Earth? What is the future of life on Earth? Are we going to keep evolving and live forever on Earth? Or is there a time limit for our survival? How life on Earth is going to end? If Earth dies, do we die with Earth or can we survive outside Earth, on different planets, on different star systems and on different galaxies? Is there a way to protect human intelligence? Can we protect, preserve, and spread human intelligence in every corner of the Universe? Charles Darwin (1809 to 1882) was the first person to answer some of those questions over 150 years ago.

I have divided this article into three parts. First, I will provide some historical background; second, I will describe how life started and will end on Earth and finally, I will describe how could we genetically engineered life to improve, protect, preserve, and spread human intelligence in every corner of the Universe.

The Impact of Darwin & Wallace on the Human Genome Project (HGP)

By his theory of Evolution and Natural Selection, Charles Darwin and Alfred Russel Wallace were the first persons to show that a relationship exists between us and all the living creatures on Earth. How true it is. Our Genome is our book of life. It carries the total genetic information that makes us. The Book of Life was conceived in our mother's womb. As we all know that we are the loving union of our parents. Our mother's egg receives our father's sperm, and we are conceived. The fertilized egg carries complete information to make us. More than seventy years ago, the Nobel Laureate, Irvin Schrödinger predicted the presence of script codes now we call the genetic code. If you examined the fertilized egg of a man, mouse, and monkey under a microscope. You find that all fertilized eggs look the same and yet only one carries the information to make a man and the second carries information to make a mouse and third carries information to make a monkey. He postulated that there exists a secret code within those fertilized egg, he called this script code now we call the Genetic Code. If we break the Genetic Code, we would be able to unlock the secrets of life. If we unlock the secrets of life, we would be able to not only create new life forms in the test tube but also be able to cure diseases by correcting the changes (mutations) and normalizing the book of life.

Schrödinger was using such a poor resolution microscope that we don't even use in our high school today. Instead, we have electron microscope today. We can magnify the same fertilized egg to a million times of its original size, almost the size of a house. What we observe inside the fertilized egg is very analogous to the house. The house has a kitchen, the cell has a nucleus. Suppose your kitchen's shelf contains 46 volumes of cookbooks which contains 24,000 chapters which carry instructions to make food for your breakfast, lunch, and dinner. The nucleus in the fertilized egg contains 46 chromosomes; 23 from our mother and 23 from our father, which carry 24,000 genes. Genes are units of inheritance which code for amino acids which perform all our body functions. Hundreds of amino acids join to form a protein and thousands of proteins interact to make a cell. Millions of cells interact to make an organ and several organs interact to make a man or a mouse or a monkey.

If the cookbook in your kitchen is written in English, it uses 26 alphabets of the English language, but the book of life of all living creatures is written in 4 letters and they are A, T, G and C. These are the initials of four chemicals called nucleotides (Adenine, Thymine, Guanine and Cytosine and are collective known as nucleotide bases. These molecules join in different proportion to generate a macromolecule which is self-replicating, self-organizing and self-evolving). Nucleotides are made of sugar Ribose (Deoxy Ribose in DNA or Ribose in RNA), a phosphate group and one of the four Nitrogen bases, two purines and two pyrimidines and the Thymine is converted to Uracil in RNA. These molecules are found in the nucleus of all living cells from a tiny blade of Grass to the mighty elephant including man, mouse, and monkey.

Darwin in the Age of DNA was proved right. Genetic analysis showed that a relationship exists between us and all the living creatures on Earth and the book of all life forms are written in the above four nucleotides. The total genetic information to make any living creature is based on four letter text and out of these four letters, only three letters called codon carries the Genetic Code for an amino acid (such as GUU is for amino acid Valine, GCU is for Alanine, GAA is for Glutamine etc.) the building blocks for all proteins. The four-letter text and three letter codes give us 64 combinations of codons. Out of 64 codons, sixty codons code for 20 amino acids and the remaining codons act as switches in which AUG serves as a start codon which codes for the amino acid methionine and which initiates DNA synthesis and the other three codons (codons UAA, UAG and UGA) serves to terminate DNA synthesis. All codons perform the same function in all living creatures, and it is not only true for ants, but it is also true for elephants. Codons for all 20 amino acids have been decoded. In many cases more than one codon codes for the same amino acid. They are called degenerate codons. All living creatures use the same genetic code. According to Francis Crick's Central Dogma a string of these nucleotides is called the DNA (Deoxy Ribonucleic Acid), stores the information. From DNA the information is transcribed into a single stranded RNA which is translated in the Ribosomes into proteins.

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. Today, we have read (mapped and sequenced the genomes of dozens of living creatures, that we were able to read) identify not only the number of genes on a chromosomes which occupy less than 2% of the chromosome, but also the total number of nucleotide bases and their order in which they are located in a species) the book of life of dozens of living creatures and found that 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. 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 relation, 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.

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 millions 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. Appearance of life on Earth is not a miracle (a rapid change). It is extremely slow process. Evolution requires accumulation of mutations over millions of years. Life evolves and nature selects. Only those variations most suited to survive in their environmental situations will survive and the rest die. Evolution must be given enough time so that the species will gradually evolve to survive. In 1973, the evolutionary Biologist, Theodosius Dobzhansky, said that "nothing in Biology makes sense except in the light of evolution." What it means is that over decades a set of evolutionary adaptations to specific selection pressures results in the evolutionary changes 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 Devine 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 ions. Mother Nature put building blocks of life together over millennia.

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. Millions of comets brought water on Earth. Seventy percent of our planet is covered with water. One wonders how microscopic life originated on early Earth. A million-lightning strikes Earth each day. At some remote corner of the Earth on a cloud of gases such as water, ammonia, carbon dioxide, methane, and phosphate rock a single lightning struck creating a macromolecule called nucleotide, an information carrying molecule. Accumulation of nucleotides resulted in RNA (Ribonucleic Acid) which is made of the same four nucleotide bases that is A-T and G-C in which Thymine is converted to a more active Uracil. RNA is a unique molecule which can store information like DNA (Deoxy ribonucleic acid) and catalyze reaction like protein. It is the very first molecule which initiates the early life on Earth. These molecules accumulated over ions giving rise to DNA 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 (from anaerobic to aerobic life) such as blue-green algae which pumped Oxygen in the atmosphere. Today, we have 80% Nitrogen and 20% Oxygen inn our atmosphere. From a single cell to multicellular life forms were evolved. Wilcock's collection of pre-Cambrian fossils such as Trilobites found in Canada showed the diversity of fossils on early Earth. Scientists wonder how they got on Earth. Using modern molecular biology technique and comparing nucleic acid and amino acid sequences across living species, 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 must conduct experiment to show how life-giving molecules appeared on Earth.

The second question is how life started and will end on Earth.

How Life Started on Earth?

In 1953, everything changed when Stanley Miller in Chicago University accelerated the natural process of creating and putting building blocks of life together. In a flask, he added ingredients of early Earth such as methane, ammonia, Carbon dioxide and Water with phosphate salts and to generate light and heat, he passed electric current. Within a week, he produced amino acids the basic building blocks of proteins. Using Hydrogen Cyanide, Ammonia and Water, John Coro and his group at the University of Houston, synthesized nucleotides in the Lab. He accelerated the natural processes and seized the power from Mother Nature. Mother Nature is too slow and too old. These scientists sent Mother Nature to a retirement home. They proved that life on Earth arose from inorganic chemical existing on Earth. Inorganic molecules can be converted to organic molecules essential for creating life. Obeying the laws of Physics, Chemistry and Biological evolution, these inorganic molecules organized themselves to become alive creating the first information molecule called RNA (Ribonucleic Acid made of the same four nucleotides A-U and G-C). Life giving RNA could be produced in the test tube without supernatural intervention.

In 1963, Leslie Orgel proposed that the information molecule RNA could similarly be synthesized from inorganic molecules which are essential for creating life. The RNA could serve as a catalyst like protein, and it could also serve as DNA (Deoxy-Ribonucleic Acid) for storing information. Information stored to create one life form in the DNA could be passed on to the next generation of microorganism. According to Darwin, repeated generation after generation of accumulation of new traits at each step of the way, nature selects the most desirable traits thus leading to the evolution of more complex organism from the simple ancestral organism. The process of converting simpler organisms to the more complex organisms takes ions.

On the Galapagos Islands, Darwin observed that eleven different islands have eleven different kinds of Finches. They have different kinds of beaks ranging from small to large and delicate to powerful. Each different type of Finch ate a different kind of food. Each Finch was particularly suited to food that was available in the environment of the island. He wondered if each Finch was born independently on each island or were they evolved from a single Finch who was blown away by a tornado or strong wind from mainland Argentina about 700 miles from these islands. He predicted that all different kinds of Finches have evolved from a single Finch. Each Finch has adapted to the environment through generations of evolution to eat the food available on the island and those Finches who could not adapt to eat the food available on the island parish.

After the completion of the Human Genome Project, we can answer the question with great accuracy and precision that Darwin & Wallace were right. Their ideas can be confirmed by the modern tools of Genetic Engineering. All the genetic tools are available to sequence (read the book of life of each Finch letter by letter, word by word and sentence by sentence) and confirm the result of the genomes of all 11 Darwin's Finches for the Genome Wide Association Studies (GWAS). The GWAS will identify each mutation responsible for all different kinds of beaks. It

was the accumulation of mutations through generations brought about the changes in the beak types. Using genetic tools such as restriction enzymes, enzymes ligase, promoters, terminators, we could cut, paste, and copy mutated genes in control Finches and could produce 11 mutated Finches with desired beak not in generations, but in weeks.

In the Age of HGP, we not only control the process of evolution, but also control the Natural Selection. Using the Genetic Engineering tool kit, we can create living creatures from non-living pieces of DNA. All living creatures have two parents, using four nucleotides and a few enzymes, Craig Venture and his group [Book by Craig Venter, Life with the speed of Light] created a living creature which has no parents. Creating artificial life opens a new field of Genetic Engineering. Now, it is possible to create new life forms which provide new food, new fuel, and new medicine to treat every disease known to mankind.

Genetic Engineering and Recombinant technology:

It involves cutting, pasting, copying, and sequencing DNA

In humans, less than two percent of the Genome codes for proteins and the remaining 98 percent of Genome contains non-coding regions which carry switches, enhancers, promoters, inhibitors etc. Only two percent of the Genome transcribes into RNA? The non-coding regions of the RNA is spliced out into mRNA which carry three letter Codons. It is the Codons which codes for amino acids and which is translated in the Ribosome into proteins. The protein carries out our body functions as soon as it folds and becomes three dimensional. As the Central Dogma of Francis Crick describes [1] that double helical DNA replicates (makes its own copies) in the nucleus and it transcribes into the single stranded RNA as it leaves nucleus as mRNA in the cytoplasm (splicing out non-coding sequence) which is translated in the Ribosomes into proteins. Information flows from both good genes and bad genes from nucleus into the cell keeping the organism healthy or sick. Good proteins from good genes keep us healthy and bad proteins from mutated genes produce bad proteins that make us sick. The flow of information is continuous and uninterrupted.

One of the greatest challenges of the 21st century medicine is how could we cut out and purify a single gene from the string of genes from the entire human genome and insert this single human gene into a biological system making a recombinants which serve as Vectors (such as bacteria or plasmids for smaller genes, and for larger genes using Phagemids, or Cosmids (Cosmid vectors are hybrids between plasmid and phage λ vectors. Cosmid vectors are designed to clone large fragments of DNA and to grow their DNA as a virus or as a plasmid. Cosmid vectors are used in homologous recombination between two different plasmids in the same cell and grown in both bacteria and animal cells).

For making their codons, we use BAC, Bacterial Artificial Chromosomes, and YAC, Yeast Artificial Chromosomes) of a single cell bacteria or yeast cell to produce large number of copies (library) of this gene. The desired gene from the recombinant library is released using restriction enzymes EcoR1 to produce large amount of highly pure protein to treat a specific disease such as producing large quantity of Insulin to treat 300 million diabetics around the world. Genetic Engineering and Recombinant technology are the answer to produce large scale other proteins such as Human Growth Hormones.

New Food:

Proteins perform all our body functions. They are made of 20 amino acids. All amino acids are made our body except eight amino acids that body does not make, and they are called essential amino acids. What are the Essential Amino Acids and why we need them? The following eight essential amino acids are: Valine, Leucine, Isoleucine, Phenylalanine, Tryptophan, Lysine, Histidine, and Threonine. We get them from outside source like meat. Without the essential amino acids, we develop a variety of diseases. Can we get essential amino acids in our diet without eating meat? Essential amino acids Codons are identified. We can insert these codons in the genomes of most consumable food like Rice, Wheat or Corn to produce the most nutritious food. The Codons for each essential amino acid and their alternative codons are described below: Valine (GTT, GTC, GTA, GTG), Leucine (CTT, CTC, CTA, CTG; TTA, TTG), Isoleucine (ATT, ATC, ATA), Phenylalanine (TTT, TTC), Tryptophan (TGG), Lysine (AAA, AAG), arginine (CGT, CGC, CGA, CGG; AGA, AGG), Histidine (CAT, CAC), Methionine (ATG), Threonine (ACT, ACC, ACA, ACG). Splicing these codons in the genomes of Rice, Wheat or Corn, we could produce the most nutritious food for the bourgeoning population of the world.

New Fuel

To run the engine of the modern society, we need electricity. To generate electricity, our power plants are fired by fossil fuels such as Coal, Petroleum, Natural gas, and Wood. By burning fossil fuel in our cars or in our power plants, we generate Carbon dioxide and oxides of Nitrogen which act as glass ceiling of a Greenhouse, and which traps sun's energy and retains heat raising internal temperature. Compared to Nitrogen gas (about 80 percent in our atmosphere) and Oxygen gas (about 20 percent) in our atmosphere, the amount of Carbon dioxide (about 0.04 percent) is negligible. Unfortunately, it is not the level of Carbon dioxide that concerns us; it is its function which acts as a glass ceiling of a greenhouse. The enormous amount of Carbon dioxide release by burning fossil fuels in cars, power plants and industrial complexes generate Carbon dioxide which spread as a thin sheet covering the entire planet which does not allow the heat to escape but trap the heat like a Greenhouse. As a result of the trapping of this heat energy, it heats up the internal atmosphere raising temperature worldwide melting polar snow resulting in sea rise. It is this trapped heat which concerns us. In a Greenhouse, we can open the windows and let this heat out. Unfortunately, we don't have such window in our planet.

Worldwide rise in temperature, could also release the trapped Methane from the permafrost and polar ice sheet frozen over millennia. Methane is another greenhouse gas which is trapped in the ice sheet since the dawn of our planetary origin. The rise in temperature decreases the ice sheet of the polar cap releasing Methane on one hand and causing the rise in sea level on the other hand. Our challenge in the next century is not only to slow down the release of Greenhouse gases but also to stop the increase level of Carbon dioxide, and to reverse the trends achieving the pre-industrial or more acceptable level. To replace the Greenhouse gases, we must develop alternative source of energy such as Wind turbine, geothermal energy, nuclear fusion, and solar panels.

Until we perfect and develop for worldwide scale of the above sources of energy, we could use biotechnology methods to generate large scale Methane as a source of natural gas. In 1996, an organism called Methanococcus Jannachil, was discovered at the bottom of the ocean floor thriving near the hydrothermal vent at extremely high temperature and pressure. It is a single cell organism belongs to the third branch of life called Archaea. It has extraordinary ability to convert Carbon dioxide (a pollutant) to Methane (a fuel). Its genome has been sequenced and almost sixty percent of its genes are unknown to science. Next generation of scientists will have to identify and isolate those specific genes responsible for converting Carbon dioxide to Methane. That gene has a start codon AUG (codes for Methionine) and will end in one of the three stop codons (UAG, UGG, or UGA). Once identified, the gene can be spliced in Chloroplast genome and harvested on industrial scale in Yeast for worldwide use.

New Medicine

The Greatest Force in the Universe is not the Nuclear Force as we used to believe, but the Force of Evolution resulting in Genetic Engineering which allows us to cut, paste and copy a gene and which bring the generation of evolution within a few days. It is the changes in mutation retained generation after generation that is responsible for evolution. Before Darwin evolution is considered as mystery of mystery. Today, evolutionary changes are brought about by mutations not over generations but in days. The process of evolution is accelerated by a new class of chemical overnight.

By controlling evolution and natural selection, we brought fundamental changes in ways of looking at all preconceived ideas about the origin of life. The hardest hit ideas are in the fields of religion, philosophy, mythology, astrology, mysticism, and magic. In the Age of Genetic Engineering and biotechnology revolution, all hypothetical concepts must either be abandoned, or it must assimilate or adapt and take a new direction that science has provided. There is no place for divine intervention in Genetic Engineering. The old system of beliefs and historical traditions must be repudiated and replaced by principles of exact science by reproducible verifiable results and by the observation of facts and by reason itself.

All living creatures have a unique ability to figure out how to solve a problem. Even a simple worm possess this ability. If you place an obstacle in its path to food, the worm figures out an alternative path to find food. A mouse is much cleverer. If you release a mouse in a most complex mazes and hide a piece of cheese in the remote corner of the maze. The mouse eventually figures out and finds the cheese. This ability to figure out solutions to problems reaches at its highest point in humans. When we first emerge Out of Africa as wonderers in search of food, water, and shelter more than three and a half million years ago; within a few generations, we figured out how to circle the globe and settle down in all seven continents. Our wondering was over when we figured out how to grow food. We started settlements around the globe when we found a piece of fertile land and grew our food. This brought the Age of Agriculture. To relief us from back breaking farming work, we figured out and developed mechanical devices to perform the farm work. This brought the Industrial Age. We split the heart of atom and learn to figure out how to convert matter into energy and run the engine of modern society. This brought the Atomic Age. Soon we figured out how to use the electrical energy to solve the most complex mathematical problem. This brought the Computer Age. We have the unquenchable thirst for knowledge. To solve complex problems instantly within seconds, we develop iPad. If we need information instantly, we pull out our iPad and search in Google, Facebook, or Twitter. This brought the Information Age.

The most important lesson we learn is to figure out and solve even more complex problems, we need more Brain Power not less. Today, more than seven and a half billion people live on planet Earth, and we are adding 90 million more people each year. Since we need more brain power to solve emerging problems; we need the quality control of the population not the quantity control of the population. There are about six thousand diseases of genetic origin caused by bad mutations. We have developed 160 genetic tests to identify deleterious mutations before a fetus is conceived. We need to develop the remaining genetic tests to ensure the quality control of the population. Most scientists don't believe in abortion; they rather believe in prevention. It is better to map and sequence the DNA of egg and sperm of the parents before conception. We found that the egg's X-chromosome is composed of a single string of 164 million of A-T, G-C nucleotide bases which carry 1,144 genes while the sperm is composed of a single string of 59 million of A-T, G-C, nucleotide bases which carry 235 genes. Using GWAS (Genome Wide Association Studies), we could easily identify which mutated genes on the sperm which are responsible for causing muscular dystrophy or color blindness in male fetus or which mutated genes in the egg is responsible for causing Hemophilia in the female fetus and replace them with the healthy egg or sperm. The old days of eugenic are over where authority makes the decision to eliminate undesirable genetic traits passing over from parents to their children. The results of the genetic analysis of the egg and sperm are presented to the parents who decide which egg and which sperm to be used for conception.

Using the tools of genetic engineering, we can figure out how to by-pass the Darwin's slow process of evolution and natural selection. The new Brain Power will soon figure out how to (work on the forbidden areas such as genes from Toxins, stem cells, Aging and Germ-line gene therapy) safely conduct Germ-line Gene Therapy not only to permanently eliminate ancestral diseases, but also to introduce traits to enhance our ability to figure out how to solve even more complex problems. The existing knowledge of Genetic Engineering is enough to replace all bad genes and replace them with good genes and produce a new generation of humans with superhuman ability. As we plan to colonize Mars during the next decade, we must figure out how to use Mars as a base to launch unmanned spacecraft's in search of exoplanets. Our aim should be to figure out how to keep humans alive after the Sun dies and how to protect, preserve, and spread human intelligence in every corner of the Universe.

With that aim in mind, we must bring revolutionary changes in our thinking. The slow evolutionary process which takes eons are now being accelerated by new scientific methods. Now, we are designing drugs that could accelerate evolution by binding to DNA causing mutation. In microbial life, we could bring mutation within 24 hours. If you add a dilute solution of any of the DNA binding alkylating agents in Petri dish growing E. Coli on Agar gel and incubate for 24 hours, you find all control colonies of E. Coli have the same circular shape growth on Agar Plate but by adding Alkylating compounds on Petri Dish show changes of colonies shapes and they appear as irregular shape of E. Coli. Alkylating compounds bind to the E. Coli DNA causes mutations within 24 hours.

How will Life end on Earth?

On February 24, 1987, a catastrophic event occurred in Magellanic cloud about 160,000 light years from here (Light travels with a speed of 186,000 miles per second, Light takes about 28 trillion miles to travel in one year called a Light year). A supernova exploded with a Titanic force. The explosion was first recorded by Stephen Sheppard of the Toronto Observatory, in Canada. Within three hours of his observation, every observatory on Earth confirmed this event. You might wonder why this event so far from Earth concerned us. The reason is that our Solar system is following the same path. It is becoming a Red Giant and then explodes as Super Nova. Our Sun has been burning for the past four and a half billion years. It burns 70 million tons of Hydrogen every second to convert to Helium. It has used up more than half of its Hydrogen content. For the first time, we realized that we are trapped in a middle age dying Solar System. As it cools, the Sun will expand and sallow the nearest two planets Mercury and Venus then become Red Giant. As it expands further, its outer rim approaches Earth. The intense heat of millions of degrees centigrade sets fire to our outer envelope of Oxygen and Ozone. Our Earth becomes a fire ball. The intense heat will boil off our oceans and melts mountains. The intense fire storm will burn char and incinerate plants, animals and kill all life forms on Earth. As the Red Giant will expand no farther, it will collapse on itself and then will explode as Super Nova destroying the entire Solar System. Does human intelligence have a future after the Super Nova explosion?

The thought of our burning to ashes in Super Nova explosion has brought humanity on a crossroad. We have an option, either to sit on our hands and do nothing, but pray and die when the Sun dies or to escape Earth before it becomes Super Nova and explode. If we do nothing, life as we

know will cease to exist. If human intelligent life extinguishes from Earth, no one in the entire Universe will ever know that intelligent life ever existed in a tiny Solar System on the third arm called the Orion Arm of the Milky Way Galaxy. The collective responsibility of every man, woman and child is to protect, preserve and spread human intelligence in every corner of the Universe.

Before 1987 Super Nova explosion, most people thought that Darwin's Theory of Evolution and Natural Selection was mindless thoughtless, and it has no purpose and no direction. Today, we know that the purpose of Darwin's work is to protect, preserve and spread human intelligence and it has a direction to take human intelligence out of this dying solar system and take it across Universe and to make human settlements in every corner of the Universe.

To save mankind from destruction, we must leave this Solar System. We must survive before the Earth dies. Our destiny lies in other Star Systems not here. Using Radio telescope, astronomers have identified at least 4000 Earth like planets called exoplanets within ten light years. As of mid-March 2018, Kepler space telescope has discovered 2,342 confirmed exoplanets and revealed the existence of perhaps 2,245 others. The total number of planets discovered by all observatories is 3,706. We need to train an army of young scientists to travel on a one-way journey in search of second Earth. We must first travel to the nearest star system called Alpha-Century which is about four and a half light years from Earth. It is a binary Solar System. It is a two-star system. It is expected that there would be more than one Earth like planets. As we develop technology to search the nearest star system, we must scan for a habitable planet in the new star system discovered by the Kepler telescope.

Kepler Telescope has the incredible ability to search for planets by measuring precisely the light coming from 150,000 stars simultaneously every half an hour round the clock. It is looking for the tiny dimming of light that is caused by passing a planet in front of one of these stars by blocking the light getting to us. Within two years, Kepler has discovered over 1,200 potential new planetary systems like ours. By studying the reflected light from these planetary systems, we learn how big the planets are and how far they are from the star. The distance is important it tells us how far the planets are from the star. If the planets are too close to the star it would be too hot to survive and if they are too far, they would be frozen. For example, in our Solar system, Venus, Earth and Mars are almost equal in size, only one of them is a good place to live. Venus is closer to Sun, and it is too hot while Mars is too far and too cold. Only Earth is hospitable to life. By analyzing the reflected light from these planets of a distant star system and comparing with the spectral analysis of Water, Ozone, Oxygen, Carbon dioxide and Methane observed on Earth, we could predict which planet in that star system is hospitable to life.

Do Humans have a future in the Universe after the Sun dies?

The answer to the above question is resounding yes. After completing the Human Genome Project, we can demonstrate how could we improve, protect, preserve, and spread human intelligence in every corner of the Universe long before the Sun dies.

To protect, preserve and spread human intelligence across cosmos, we must follow the advice of Daniel Golden who was the former director of NASA and one of the foremost intellectuals of this century. He provided a vision to explore the fate of humans on Earth. When we were about to complete the Human Genome Project, he addressed the scientists of both US agencies that is NIH (National Institutes of Health) and NASA (National Aeronautics and Space Administration). He challenged scientists in both agencies. In an address to both agencies, he said that he had a dream to land men on Red Planet Mars before this century is over. Not just land men on Mars, but to colonize Mars and use Mars as a base to launch unmanned spacecraft's in search of Earth like planets, a second Earth, to transport humans in new environment. To succeed in this endeavor, he proposed three assignments for scientists at NASA to work on spacecraft's and three assignments for scientists at NIH to work on astronauts. First, NASA challenge is (1) using the hardest and lightest material (like Graphene from Graphite) to build a fleet of city-size spacecraft's to transport humans to other star systems; next (2) using fusion reaction, build an unlimited source of energy (recently done by the Plasma Lab in Princeton University where they successfully fused Hydrogen atoms to generate Helium atoms and produced fusion energy and (3) using supersonic technology gain speed at least half the speed of light.

Within that short period, NASA accomplished miracles. On December 18, 2021, NASA is to launch its most prized telescope, the James Webb Space Telescope, the most powerful telescope ever constructed to study Universe as it was soon after the Big Bang, 13.72 billion years ago. It is 100 times more powerful than the Hubble telescope. It will be placed in an orbit a million miles from Earth. It will not only be able to see billions of light-years away, but it will also be able to study the atmospheric composition of other planets

While scientists at NASA will be working on spacecrafts, scientists at NIH are asked to work on the Astronauts and are given the following three assignments: Dr. Golden said he cannot send astronauts on a long one-way journey in search of second Earth who is suffering from pain. He asked us to identify genes which are responsible for causing pain and suffering. Second, he said that he cannot send astronauts on a long journey who is suffering from diseases. He asked us to identify all genes which are responsible for causing diseases and finally, he cannot send astronauts on a possible one-way journey if they live only a hundred years. He asked us to find genes which are responsible for aging. Within less than ten years of his speech, I am pleased to report that we have made enormous progress in all three areas. We have identified a master mutated endorphin gene which controls a network of genes responsible for causing pain and suffering. We have identified over six thousand genes which are responsible for causing diseases. Now, we can design drugs to shut off these genes. The most important of accomplishment of this century is the discovery of aging gene which could double human age and for which Elizabeth Blackburn and Carol Greedier shared the 2009 Noble Prize in Medicine. For future deep space travelers, we need to prolong healthy life.

As I said above, while NASA is working on improving spacecrafts, we at NIH will work on Astronauts. We are the largest biomedical center in the world. More than 21,000 scientists are working in more than 3,000 Labs. To study the entire Universe, Physicists work on a single atom. To study a human, we at NIH work on a single cell. Life begins with a single cell. The nucleus of a single cell carries instructions to make all life forms from a tiny blade of grass to mighty elephant including man, mouse, monkey and microbe.

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; and 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 genes responsible for six thousand diseases and two thousand Pseudo-genes that have lost their functions, during evolutionary time.

Our entire book of life is written in four genetic letters called nucleotides and they are A (adenine), T (thymine), G (guanine) and C (cytosine). These are the initials of four chemicals are called nucleotide and they are found in the nucleus of all living cells including humans, plants, and animals. Nucleotides are information carrying molecules. Instruction in a single gene is written in thousands of AT/GC base pairs that are linked together in a straight line and we call them DNA (Deoxyribose Nucleic Acid) - Nobel prize was awarded to Crick, Watson & Morris Wilkins [1] for discovering the double helical nature of the DNA structure which is transcribed into a single stranded RNA (after splicing out the non-coding nucleotides, the RNA is converted to mRNA in which the less water soluble methyl group in Thiamine, T, is converted to more water soluble Uracil, U, by replacing Methyl group with a Hydroxyl group) which leaves the nucleus into Cytoplasm where it is translated in Ribosomes into Amino Acids leading to proteins). When thousands to millions of AT/GC base pairs contain information to make a single protein, we call that portion of AT/GC base pairs a gene (Nobel Prize was awarded to Khorana & Nauenberg for making a functional gene). Out of four nucleotides, three nucleotide code for an amino acid called a Codon. Four letter nucleotide text, code for all 20 amino acids in 64 codons.

The starting Codon for a gene is AUG which codes for the amino acid Methionine after several thousand Codons for different amino acids, comes the stop codon. There are three stop Codons, and they are UGG, UGA, UAG. After the stop Codon appears, no more amino acids are added, and DNA synthesis stops. If we count all the AT/GC base pairs in a single cell of our body, we will find that there are 3.2 billion pairs of bases present in every cell from each parent. The entire AT/GC sequence of six billion four hundred million base-pair is called the Human Genome or the book of our life which carries total genetic information to make us.

As I said above, we deciphered all 46 chromosomes. What surprise us most was 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 non-coding 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 bases. Before sequencing (determining the number and the order of the four nucleotides on a chromosomes), it is essential to know how many genes are present on each chromosome in our Genome.

In 1990, US Congress authorized three billion dollars to NIH to decipher the entire human book of life under the title The Human Genome Project. We read the entire book of life letter by letter, word by word and sentence by sentence, all 46 chromosomes containing six billion four hundred million nucleotide. It was published in the Scientific Journal Nature and was linked to website for the world to see. If you have access to a computer keyboard, you have access to all the information.

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 nucleotides bases and it has only 2,610 genes. The chromosome-2 contains 255 million nucleotides bases and has only 1,748 genes. The chromosome-3 contains 214 million nucleotide bases and carries 1,381 genes. The chromosome-4 contains 203 million nucleotide bases and carries 1,024 genes. The chromosome-5 contains 194 million nucleotide bases and carries 1,190 genes. The chromosome-6 contains 183 million nucleotide bases and carries 1,394 genes. The chromosome-7 contains 171 million nucleotide bases and carries 1,378 genes. The chromosome-8 contains 155 million nucleotide bases and carries 927 genes. The chromosome-9 contains 145 million nucleotide bases and carries 1,076 genes. The chromosome-10 contains 144 million nucleotide bases and carries 983 genes. The chromosome-11 contains 144 million nucleotide bases and carries 1,692 genes. The chromosome-12 contains 143 million nucleotide bases and carries 1,268 genes. The chromosome-13 contains 114 million nucleotide bases and carries 496 genes. The chromosome-14 contains 109 million nucleotide bases and carries 1,173 genes. The chromosome-15 contains 106 million nucleotide bases and carries 906 genes. The chromosome-16 contains 98 million nucleotide bases and carries 1,032 genes. The chromosome-17 contains 92 million nucleotide bases and carries 1,394 genes. The chromosome-18 contains 85 million nucleotide bases and carries 400 genes. The chromosome-19 contains 67 million nucleotide bases and carries 1,592 genes. The chromosome-20 contains 72 million nucleotide bases and carries 710 genes. The chromosome-21 contains 50 million nucleotide bases and carries 337 genes. Finally, the sex chromosome of all females called the (X) contains 164 million nucleotide bases and carries 1,141 genes. The male sperm chromosome contains 59 million nucleotide bases and carries 255 genes.

If you add up all genes in the 23 pairs of chromosomes, they come up to 26,808 genes and yet we keep on mentioning 24,000 genes needed to keep us function normally. 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].

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 pseudo genes. 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 don't 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 Pseudo genes. Recently, some Japanese scientists have activated the pseudo genes, this work may create ethical problem in future as more and more pseudo genes are activated.

We all carry 220 different tissues in our body and yet we have a single genome that is the same DNA in every cell. How can all cells carry 24,000 genes and have the same DNA made of AT and GC nucleotides and yet they function in all 220 different tissues? The answer is not all 24,000 genes function in every cell of our body at the same time. Epigenetic answers one the most important questions in the cellular evolution. Small fraction of genes function in different organs and the rest are turned off by either Methylation or Acetylation which serves as Epigenetics agents. For Methylation and Acetylation, the common reagent in the Lab is Dimethyl sulphate or Diazomethane in Sodium Hydroxide for Methylation and Acetic Anhydride in Sulfuric Acid for Acetylation. The common Epigenetic agents in our body are Folic Acid responsible for Methylation and Acetyl Choline acts as Acetylating Agents. They can Alkylate or Acetylate both DNA and Histone proteins shutting off genes either temporarily or permanently. Methylation is a common and widely used mechanism for Epigenetic modifications in cells. Abnormal mutations in the Epigenome have been shown to be correlated with many human diseases, including different cancers, autoimmune disorders, neurological disorders (Fragile X syndrome as well as Huntington, Alzheimer, and Parkinson diseases including Schizophrenia).

The Sequencing of the Human Genome which is not only reading the entire book of life of human being letter, by letter, word by word and sentence by sentence, chapter by chapter but also the order in which these letters are arranged called sequencing, is the greatest discovery of all times. The sequencing of the Human Genome will answer the most fundamental questions, we have asked ourselves since the dawn of human

civilization; what it means to be human; what is the nature of our memory, our conscientio usness; our development from a single cell to a complete human being; the biochemical basis of our senses; the process of our aging; the scientific basis of our similarity and dissimilarity. Similarities that all living creatures from a tiny blade of grass to the mighty Elephants including man, mouse, monkey, and microbes are all made of the same chemical building blocks and yet we are so divers that no two individuals are alike, even identical twins are not identical; they grow up to become two separate individuals.

By examining and comparing the sequences of thousands of abnormal and normal genomes of egg and sperms, we can identify all mutated genes in a genome. Each of us carries a single copy of half a dozen mutated genes. We are a carrier of one copy of the bad gene, if we marry closely related person who is bringing the other copy of the same mutated gene; the fetus is affected. Related couples in which both parents are the carriers of the same mutated gene; they are most likely to have children who inherit both recessive copies of the same bad genes. Such couple is most likely to have a baby which come down with horrendous genetic diseases and they are most likely to terminate the pregnancy. Although it is a painful decision, it is better than watching their children suffer and die of a terrible disease.

If the fetus carries both bad copies, it will be severely sick. Let me explain with an example how this work will help parents to decide to have a baby even before conception or during pregnancy. A newlywed couple could either conceive a baby either in the bedroom or in the test tubes. If there is a family history of a disease, it is advisable to have in vitro fertilization. The couple gives a sample of eggs and sperms for genetic analysis before conception. Detection kits for several hundred genes are already being developed. The test result may show that the sperm is carrying a genetic defect on Y-chromosome that will make the baby a color blind or give him MS (muscular dystrophy). Doctors will inform the parents whether the child will be incurably blind, or carry a gene for defected heart, kidney, or liver. During the ancient times when Eugenic was at its peak, the authority makes the decision about the fate of the fetus. These days, Parents make the decision to bring this child into this world. How many parents will love to have a blind or permanently sick child in their families? Not many. We must run the census among our people to get the results. It seems reasonable to assume that most parents will not be able to care for that fetus. We may not be able to correct that defect tomorrow, but day after tomorrow may be or in some distant future. We will be able to correct that defect at an enormous cost. Is there any reason for poor parents to use that defected fertilized ovum and let it grow to full term at an enormous medical expanse? I am sure some rich parents will love to have children at all costs. Such children of rich families will not be burden on society or on our health care system. Since completing the Human Genome Project, out of six thousand mutated genes, we have already developed over 1500 tests to identify mutated genes; we can provide in vitro fertilization (IVF) to ensure that the fertilized egg is free from all genetic defects. Instead of having conceived children in the bedroom, couples will be able to select out the very healthy eggs and sperms and fertilized them in the test tube and implant them in the mothers. This way we can have the quality control of the babies we bring into this world. The quality control of the population could be accomplished by in vitro fertilization only. About 25,000 Mendelian diseases (single gene defects) have been identified and approximately ten thousand are confirmed to specific genes. Developing novel drugs to treat those diseases is expensive and time consuming.

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

Human Genome contains a catalog of traits written on genes in nucleotide sequence. Our Genome also provides a catalog of all 24,000 genes; it also provides the number and location of each gene on the chromosome. The catalog also identifies 16,000 good genes, 6,000 bad genes and 2,000 pseudogenes (they lost their function).

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 genes 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, you can break off the information flow and stop having children or stop donating mutated eggs and sperms.

Our Genome is not just a diagnostic road map of our genes, it tells us how 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 the production of Insulin and Human growth hormone. On the other hand, identifying the bad genes and tell us how to design drugs to shut off bad genes responsible for causing Cancers, Cardiovascular disease, and Alzheimer. We have already demonstrated that using the genetic engineering techniques, we can cut, paste, copy, and sequence a good gene for industrial scale preparation such as Insulin to treat 300 millions of diabetic around the world.

Once the good and bad genes are identified, we know that the good genes codes for good proteins which keep us healthy, and the bad genes produce bad protein that make us sick. Genome sequencing of bad genes start a new era of Genomic Medicine which is based on designing drugs 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. How do we design drugs to treat these diseases?

Historical Background for Using Nitrogen Mustard for Treating Cancer

Fitz Haber, a German Army officer, worked on the development of Chemicals as a Weapon of War. He was responsible for making deadly Nerve gases and Nitrogen Mustards. Before the WWI, he was honored with a Nobel Prize for capturing Nitrogen directly from the atmosphere for making Nitrate fertilizers by burning the element Magnesium in the air forming its Nitride. Upon hydrolysis, Nitride is converted to its Nitrate. Using this method, we could make unlimited amount fertilizer. Nitrate is also used for making explosive. Soon after the WWI, Haber was charged with a crime against humanity for releasing hundreds of cylinders of Chlorine gas on the Western front killing thousands of soldiers in the trenches. When Allied forces 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.

Nitrogen Mustard was mercilessly used 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 a success. 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 Merophan. [7-13]. 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 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.

Rationale for Developing Nitrogen Mustard Analogs as Anti-Cancer Drugs

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 to make its Hydrogen-Chloride salt. Nitrogen Mustard Hydrogen Chloride salt is separated. No matter how much precautions you take, after the experiment, if you would take an alcohol swab of walls, doors, knobs and run a mass spectra of 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, will anyone approve this study in the University Research Lab, probably No one. 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 larger the ratio the more toxic to cancer cell. When tested against Walker Carcinoma 256 in Rats, most Nitrogen Mustards analogs cross-link both strands of DNA and give a CI of ten.

Rationale for Developing Aziridine Analogs as Anti-Cancer Drugs

Radio labeled study showed that Nitrogen Mustard shut off genes by binding to DNA by cross-linking both strands of DNA. We also discovered that radio-labeled Nitrogen Mustard does not bind to both strand of DNA simultaneously. First, one arm of the Nitrogen Mustard binds to a single strand of DNA, the Carbonium ion of the second arm is so reactive, it attacks its own Nitrogen atom forming a three-member intermediate ring called the Aziridine ion. Aziridine is extremely unstable in the acidic medium. As the living cells grow, they use glucose as a source of energy. Glucose breaks down to Lactic Acid which opens the Aziridine ring generating a Carbonium ion which attacks the second strand of DNA by cross-linking both strands. Once cross-linked, the two strands of DNA do not replicate. The cancer cell dies. On the other hand, Aziridine binds to a single strand of DNA. During cell division, the two DNA strand separate and the cell replicates. We thought that Aziridine analogs would be less toxic to normal cells and more toxic to cancer cells. It is indeed found to be true. We made a series of Dinitro Phenyl Aziridine compounds to test against the experimental tumor Walker Carcinoma 256 in Rats. One compound the benzamide of Dinitrophenyl Aziridine (CB 1954) gave the CI of 70 highest to cancer cells ever recorded [14, 15].

As I said above, in the Laboratory of Professor Ross, I had worked with the deadliest Nerve agents making their derivatives such as Nitrogen Mustards, Carbamates and Aziridines developed during Hitler's time for evil purposes. We converted the evil chemicals into good chemicals. These agents easily pass-through various layers of our skin from Ectoderm to Mesoderm to Endoderm. They easily enter the cell nucleus destroying the beta and gamma cell which develop immunity. Then they enter the nuclear membrane where they find the stem cells. Stem cells differ from say skin cells. In Stem cells all 24,000 genes are functioning, cells have not yet differentiated. On the other hand, differentiated cells like skin cells which are differentiated, the Epigenetic groups such as methyl group or Acetyl group have shut off all other genes except the skin cell genes.

While Professor Ross worked with the Nitrogen Mustard by cross-linking both strands of DNA, as his Doctoral student, I was assigned to work with Aziridines which binds to a single strand of DNA. As a part of my Doctoral Thesis, I attached alkylating Aziridine which acts as prodrug, to dyes like Dinitro Benzamide to attack the DNA of an experimental animal tumor called Walker Carcinoma 256. As I said above, the cancer cells grow faster than normal cells, they use more Glucose as a source of energy. Glucose breaks down to produce Lactic Acid. The Aziridine moiety is activated in acidic solution. The Aziridine breaks down to open its Aziridine ring to produce a positive Carbonium Ion. The Carbonium ion is extremely reactive; it binds to a single strand of DNA. It preferentially binds to N-7 of Guanine killing the tumor cells. Professor Ross and I have demonstrated the attack on N-7 of Guanine using the radio labeled studies. As a part of my Doctoral and Postdoctoral studies, over the years, I made 120 Dinitro-Benzamide derivatives for testing against Walker Carcinoma 256 in Rats [16].

From our Labs at the Royal Cancer Hospital, University of London, England, I had sent NIH (National Institutes of Health) over 120 drugs for NCI (National Cancer Institute) screening program. NCI honored me with the Fogarty International Fellowship Award to come to America to continue my work with Aziridines translating the animal work in Humans. As I said above, NIH is the largest biomedical center in the world. It has unlimited facilities (chemicals, equipment, and personnel). Twenty-one thousand best and brightest scientists selected from Ivy League schools work in 26 institutes in more than three thousand labs. I was honored to join this group at NCI. The completion of the Human Genome Project at NIH has helped us identify all six thousand mutations responsible for causing diseases.

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.

Reference sequence

We can scan the whole genome (Reference Sequence) for its response to a given situation. When we look at the sequence of a normal cell and compare with the sequence of an abnormal cell, we see the defects. Or when we compare the patient's sequence with the reference sequence, looking for the gene expression of a specific proteins, from a specific genes and for a specific nucleotide sequence, we can identify a specific mutation which identify a specific disease.

In the olden days, before sequencing human genome, if a patient sees a Physician with several complains such as he feels dizzy, have headaches, can't focus, feels sleepy, doesn't remember, can't recall. The physician would say to his patient, I don't know what is wrong with you; I will order several tests to see if any of these tests show if my guess is right and if he guesses wrong, he will throw those tests and order few more tests to see if he could identify the illness. The guesswork diagnosis is expensive and time consuming and the trial-and-error days are over. **Now, after**

sequencing the human genome, the physician would say I don't know what is wrong with you, but I do know where to find it. It is written in your Genome. Let us sequence your genome. It would be easy for a Physician to scan the patient's entire genome and compare against the Reference Sequence to identify the mutations responsible for causing the symptoms. We will take a small blood sample of 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, we can easily identify the mutations responsible for causing the symptoms. The result will provide the best diagnostic method to identify a disease. The accuracy of this diagnosis is confirmed by comparing with the 1000-genome project.

After examining the patient's genome sequence, the physician identifies genetically inherited mutations on five chromosomes. 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 predict the early signs of brain cancers, the Glioblastomas. 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. Most patients die within 14 months of diagnosis. Patients who have a family history of Glioblastomas, needs to sequence their egg and sperm for any deleterious mutations. After sequencing egg and sperm, if not stop at the conception, these mutations will be passed on to the next generation.

All known Glioblastomas causing genes are located on five different Chromosomes and carries a total of 9,579 genes. It appears impossible to treat Glioblastomas since we don't know which nucleotide on which gene and on which Chromosome is responsible for causing the disease. With the completion of 1,000 Human Genome Project, it becomes easier to identify by simply comparing the patient's Chromosomes with the one thousand genomes, letter by letter, word by word and sentence by sentence; we could identify the 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 design drugs to treat Glioblastomas.

I used the same rationale to continue my work in America. I brought the idea from London University of attacking one strand of DNA using Aziridine, but I do not want to use the same dye Dinitro benzamide. One day, I heard an afternoon lecture at NIH in which the presenter described that methylated radio labeled Quinone crossed the Blood Brain Barrier (BBB). When injected radiolabeled Quinone, within 24 hours, the entire radioactivity was concentrated in the Brain of mice. I knew that Glioblastoma multiforme, the human brain tumor, is a solid aggressive tumor like Walker Carcinoma in Rats. I decided to use Quinone moiety as a carrier for Aziridine rings to attack Glioblastomas. In my previous work at the London University, by introducing just one Aziridine and one Carbamate moiety to Dinitro Benzine ring, I produced enormously toxic compound. When tested against Walker Carcinoma in Rats, it was so toxic that its toxicity could not be measured. All animals died. For safety reason, further work with multiple Aziridines and Carbamates moieties at the London University was discontinued.

Fortunately, the Quinone ring is not only cross the Blood Brain Barrier (BBB), but also it is much safer to use. I could introduce different combinations of Aziridine rings and Carbamate moieties and could create havoc for Glioblastomas. The analogs of Aziridines and Carbamates Quinones are prodrug that is they remain inactive in neutral spinal fluid medium. Once they cross the BBB, they become activated when the tumor start growing. On the Quinone ring, four substitution sites are available. I could introduce various combinations of Aziridine rings and Carbamate moieties and could create havoc for Glioblastoma. My major concern was how toxic this compound would be to the brain cells. Fortunately, brain cells do not divide, only cancer cells divide. To grow, cancer cells use glucose as a source of energy. Glucose is broken down to produce lactic acid. It is the acid which activates the aziridine and carbamate generating Carbonium ions attacking Glioblastoma. Of all the over two dozen combinations of Aziridine and Carbamate, the most active analog was Diaziridine, dicarbamate Quinone, I named it AZQ. On treating with AZQ, the rapidly growing Glioblastoma start shrinking. Radiolabeled C-14 Aziridine will identify which nucleotide on which genes and on which Chromosomes of Glioblastomas is attacked by AZQ [17-20].

Over the years, I 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 US Government (US Patent 4,146,622 & 4,233,215)). One of them called AZQ acts as a silver bullet. Glioblastoma was not only stop growing, but also start shrinking. For the discovery of AZQ, I was honored with the "2004 NIH Scientific Achievement Award" one of America's highest award in medicine and I was also honored with the "Vidya Ratna" a Gold Medal, one of India's National Medal of Honors. (Exhibit # 1, 2, 3 & 4).

2004 NIH Scientific Achievement Award

Presented to

Dr. Hameed Khan

By

Dr. Elias Zerhouni,

The Director of NIH

During the NIH/APAO Award Ceremony held on December 3, 2004.

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

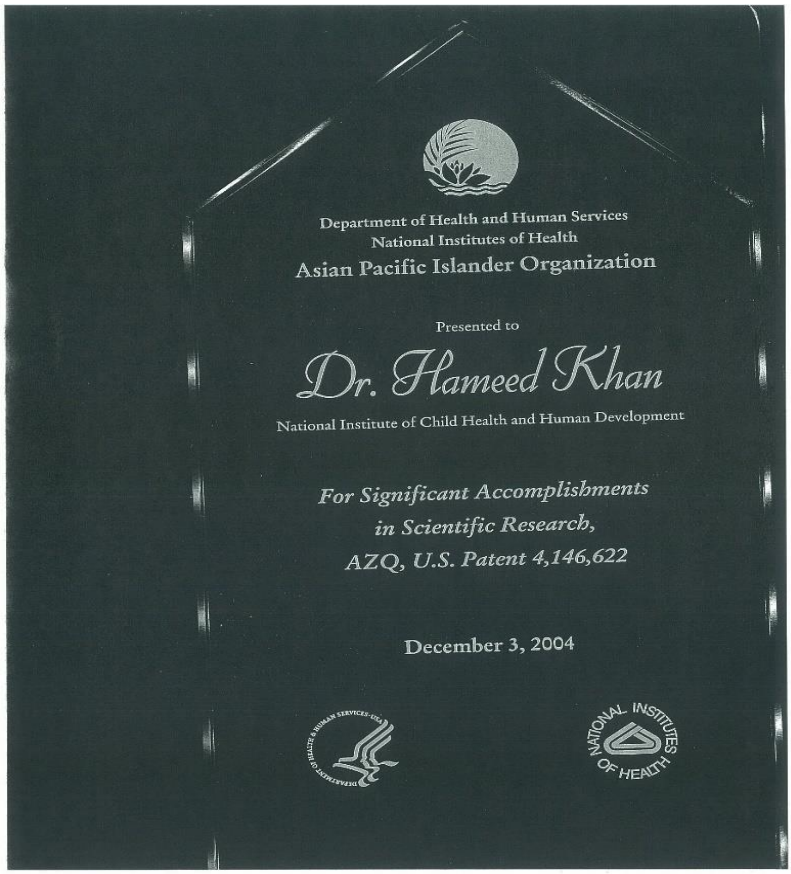


Exhibit # 2

His Excellency, Dr. A.P.J. Abdul Kalam,

The President of India

Greeting

Dr. A. Hameed Khan,

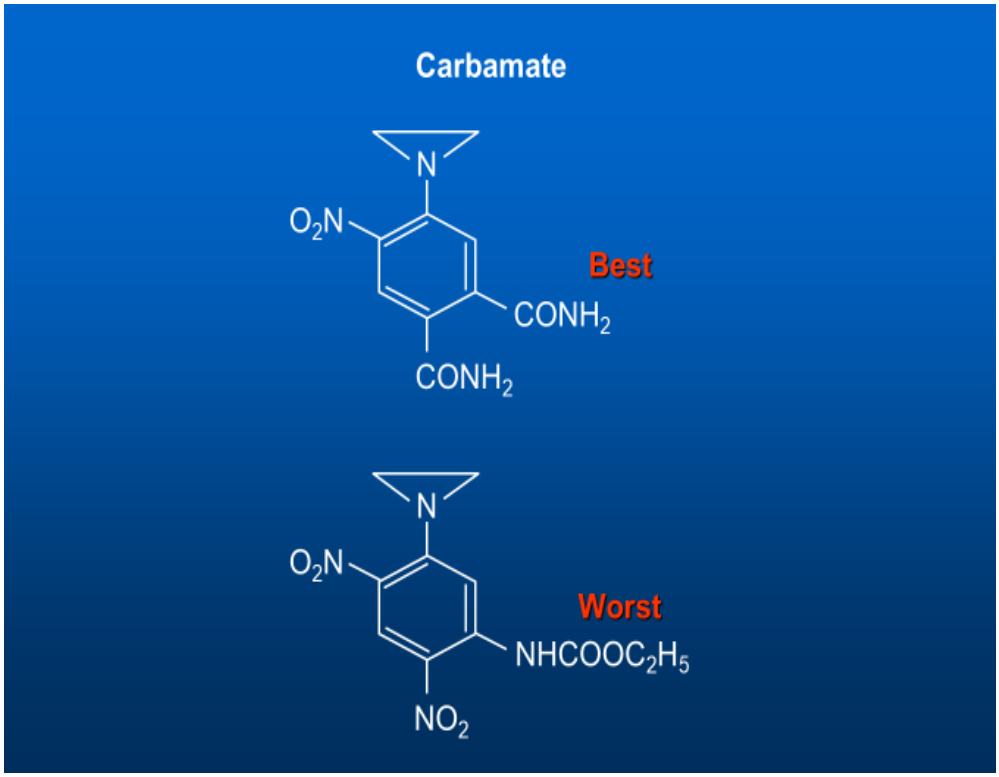
Discoverer of anti-cancer AZQ, after receiving 2004, Vaidya Ratna,

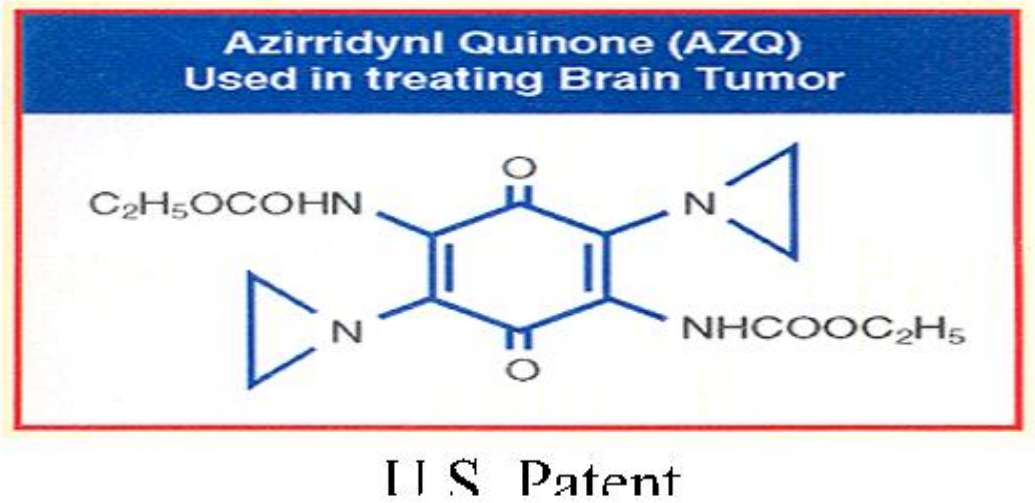
The Gold Medal, One of India's Highest Awards in Medicine

At The Rashtrapathi Bhavan (Presidential Palace), in Delhi, India, during a Reception held on April 2, 2004.

Exhibit # 3

Single Strand DNA Binding Aziridine and Carbamate





U S Patent

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.

What other cancers should we explore next?

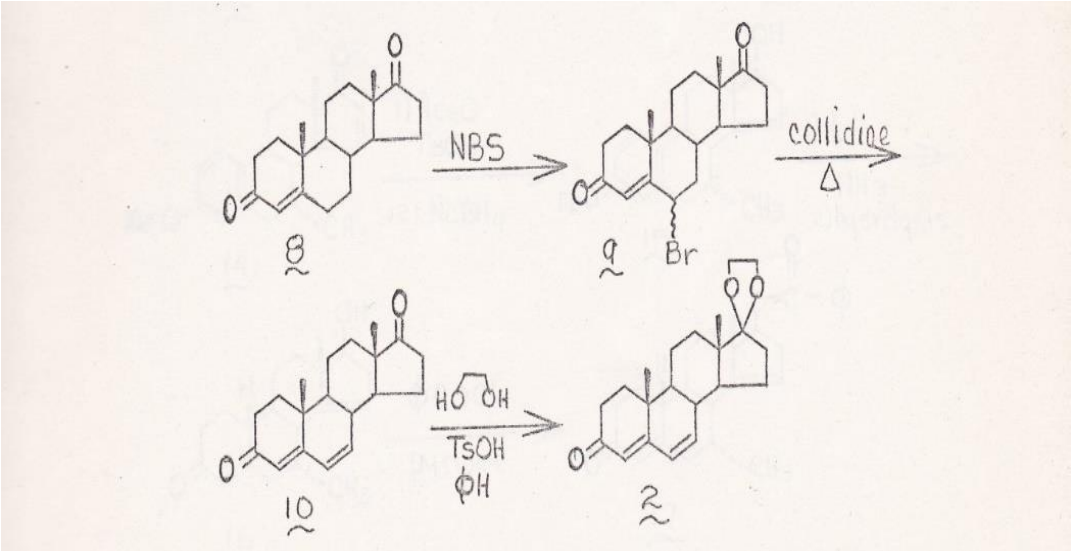
Could I use the same rationale for treating Breast and Prostate tumors?

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. Once AZQ is developed to protect the brain, I could focus on the Breast and Prostate Cancers.

Now, we found out that using Nanopore Sequencer we could rapidly sequence the genome of the Brest cancer and by comparing with Reference Sequence, we could identify all mutations on Chromosome-17. By taking monthly MRI [22], we could see the progress of the Breast Cancer. Before the Breast Cancer spreads to other organs, we could develop novel drugs to treat the Breast Cancer.

Radiolabeled studies showed that male hormone Testosterone has great affinity for female Breast, Ovary, and Fallopian tube cells. On the other hand, Estrogen, the female hormone, has great affinity for male prostate gland. By attaching multiple Aziridine rings and Carbamate ions to both Hormones, I could attack the Breast and the Prostate cancer.

In a Breast tumor, within the start and stop codon, BRCA1 gene has captured over two hundred thousand nucleotide bases. The BRCA1 genes carries about three thousand mutations. These mutations are caused by radiations, chemical or environmental pollutants, viral infection, or genetic inheritance. To attack the mutated nucleotides among the three thousand cells in BRCA1 gene, I could use male hormone, Testosterone, and bind multiple radio labeled Aziridine and Carbamate ions to attack BRCA1 mutations. By using MRI, I could show how many radio-labeled nucleotides were bound to which mutations. 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.



It was Carl Djerassi (23) who 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 ions on 10 positions which could be de-brominated by Collidine to introduce a 9, 10 double bond which could further be brominated to produce 9, 10 dibromo compound. To enhance its toxicity to cancer cells, both dibromo ions could be replaced either by one aziridine and one carbamate or two aziridines and two carbamate ions. I could further increase or decrease the number of Aziridines and Carbamates ions to get the maximum benefit by further brominating position 15 and 16 to introduce additional Aziridine and Carbamate moieties. The derivatives of Aziridines and Carbamates are prodrug. They remain inactive in neutral pH, but as soon as the cancer cells start dividing, it uses Glucose as a source of energy. Glucose is broken down to Lactic Acid. As in AZQ, the acid activates the Aziridines and Carbamate moieties; they are broken down to generate the powerful carbonium ion which attack the cancer cells.

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

Conclusion

Had Darwin live today, he would have been truly proud of our accomplishments. Within 150 years of his theory of evolution by natural selection, we are ready to take his ideas across the Universe. **It was Darwin's theory of evolution and natural selection that gave our ancestors human intelligence and human conscientiousness. They had little knowledge and understanding of survival when they came out of Africa about three million years ago, and within seven thousand generations, they walked around the world and settled down on all seven continents. They not only, circumnavigated the world; they climbed the tallest mountain and gone the bottom of the deepest ocean; they split the heart of atom and walked on the surface of the Moon and came home safely. Our next step in the search for life in the cosmos is our dream to create settlements on the surface of Mars and using Mars as a base to launch unmanned spacecrafts in search of habitable planets in distant star systems. Darwin's evolutionary ideas taught us that the future can be greater than the past. Fifty-five years ago, we touched greatness when we walked on the surface of the Moon and that was a turning point in history. There was a time when we soar to the Moon now, we must dream again to soar to the Mars and soar to explore thousands of exoplanets in the Universe.** If we don't destroy ourselves by going to Nuclear War or Environmental Collapse, within a million year, we will have human settlements on distant star systems. Humans have future in Cosmos after the Sun dies and we can protect, preserve, and spread human intelligence in every corner of the Universe.

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