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Review Article

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The Impact of Sequencing Human Genome on Treating Mental Disorders Including Brain Cancer

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Abstract

The purpose of this abstract is to study mental disorders that tend to run in the families, suggesting potential genetic roots. If you inherit these defected genes, there is nothing you can do to prevent these diseases. Such illnesses include Aautism, Attention deficit hyperactivity disorder (ADHD), bipolar disorder, major Depression, Epilepsy, Huntingdon, Parkinson, and schizophrenia. Developing novel drugs to treat mental disorders present the greatest challenge because our brain is covered by a protective fatty membrane called the Blood Brain Barrier (BBB). The BBB filters out all toxic chemicals except highly toxic and highly addictive narcotics. Of all the non-toxic chemicals tested to cross BBB, only Quinone is a non-toxic and non-addictive molecule that crosses the BBB with great ease. In making AZQ (US Patent 4,233,215) to treat Glioblastoma, the brain cancer, we used Quinone as a carrier to transport across BBB highly toxic Aziridine and Carbamate moieties to attack DNA of growing brain tumor like Glioblastomas. The active moieties of Aziridines and Carbamates act as prodrugs. They remain inactive in the brain in neutral and basic spinal fluid but are activated in acidic environment. The growing Glioblastoma tumor uses Glucose as a source of energy which is broken down to produce Lactic acid. It is the acid that activates the prodrug releasing highly reactive Carbonium ions which attack the Glioblastoma tumor DNA shutting off the genes controlling the tumor growth. Although it works for Glioblastoma, unfortunately, in other mental disorders described above, the prodrug is not activated due to the absence of acid. The genes responsible for causing this mental disorder are not shut off. It is the challenge for the next generation of scientists (my students) to activate the prodrugs in the absence of acid to treat all other mental disorders.

Keywords: Mental Disorder, Glioblastoma. BBB, Aziridine, Carbamate, AZQ

A Note to my Readers

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

Special Notes

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

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

Introduction

The essence of life is information, and the information molecules are called the nucleotides: they are located on the long string of molecules called chromosomes. There are four similar nucleotides, and they are Adenine (A), Thiamine (T), Guanine, (G) and Cytosine (C). A string of nucleotides is called DNA (Deoxyribose Nucleic Acid). We are the extension of the same single DNA molecule that was formed nearly four billion years ago. The book of life in all living creatures from the microscopic life form to the present-day humans is written in the same language of DNA using the same four nucleotides. This is the evidence-based information provided by the evolutionary biology. The total genetic information that makes us human is called the Human Genome.

Our journey began with the simplest single cell life when our mother's egg receives our father's sperm, we are conceived. The fertilized egg attaches itself to our mother's womb. It draws nourishment; it grows, divides, multiplies, differentiates and in nine months we are born as a complete human being. By the time we are matured that single cell has replicated over a trillion time. Each cell carries the same information as the first cell. Millions of cells interact to make a tissue and there are 220 different tissues that interact to make an organ and several organs interact to make a human. During development, if growing fetus is exposed to toxic chemicals, (either by smoking or drinking), radiations, viral infections or genetic inheritance, the newborn baby is most likely to be abnormal. If we read the book of life of the fetus (sequencing its genome), it will help parents to decide if they want to terminate the pregnancy. To determine if the growing fetus is healthy and not carrying any serious genetic defects, it was realized that we must develop technology to read his book of life that is to sequence his genome. Sequencing began with reading the book of life of simpler creatures such as viruses and bacteria.

In 1977, Fred Sanger was the first person to sequence the entire genome of the simplest virus called bacteria phage phi X 174. It has only nine genes arranged on a single chromosome made of 5,375 base pair. It spells out in term of AT and GC nucleotide base pairs.

The pace of sequencing of other forms of life accelerated the next milestone was reached in late 1995, when the first gene map of an entire haemophiles influenzas genome was sequenced which contained 1,743 genes located on a single circular chromosome made up of one million eight hundred, thirty thousand and thirty-seven base pairs. In early 1960 even this Herculean feast was surpassed by when the genome of ordinary baker's yeast was decoded. It contains 12 million base pairs divided into six thousand genes on sixteen chromosomes. Yeast is particularly important it shares so many of its genes with humans.

In 1997, scientists at the University of Wisconsin, Madison, announced that they have unravel the genome of the bacterium E. coli which contains four million six hundred thirty-eight thousand eight hundred and fifty-eight base pairs and carries four thousand and three hundred genes. Fifteen percent of the human gene sequence contains part of the E. coli genome. Presently, scientists working on many fronts performing DNA sequencing of a variety of organisms simultaneously. We expect to see scientists in the next few years announcing the DNA sequencing of increasingly complex organism. In summary, using Sanger's method, other groups started sequence the genome of more complex living species and found that a common virus genome is made of 0.1 million base pairs; Yeast is made of 12 million base pairs, Fly Drosophila is made of 180 million base pairs, Tomato is made of 700 million base pairs, mouse is made of 3 billion base pairs. The greatest challenge was to sequence the entire human genome made of 3.2 billion base pairs from mother and 3.2 billion base pairs from father. A total of 6.4 billion base pairs spread over 46 chromosomes.

The Human Genome Project

"Know Thyself'

This phrase was spoken by Socrates more than 2,300 years ago. It was carved into stone at the entrance to Apollo's temple at Delphi in Greece. Socrates believed that the first step to true wisdom is to "know thyself" because only then can one appreciate what one understands and what it remains to be learned. Aristotle agreed that "Knowing yourself is the beginning of all wisdom." For millennia, we had been constantly striving to achieve self-knowledge. Now, we found the answer to the phrase by sequencing human genome. Everything we want to know about ourselves is written in our genome.

As I said above, the entire book of life of all living creatures on Earth is written in four genetic letters called nucleotides. These nucleotides are found in the nucleus of all living cells including humans, plants, and animals. 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 for discovering the double helical nature of the DNA structure which is transcribed into a single stranded of RNA (in mRNA the less water soluble methyl group, Thiamine, (T), is converted to more water soluble Uracil, (U), by replacing Methyl group with a Hydroxyl group) which leaves the nucleus and moves into Cytoplasm where it is translated in Ribosomes into Amino Acids leading to proteins) [1]. 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). 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 the nucleus of every cell. The entire AT/GC sequence of 3.2 billion base-pair is called the Human Genome or the book of our life which carries total genetic information to make us. The reading of the total genetic information that make us human is called the Human Genome.

In 1990, US Congress authorized three billion dollars to NIH (my institute) 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. This is how we began our work on sequencing human genome.

As you know, you and I are the loving union of our parents. Our mother's egg receives our father's sperm, and we are conceived. The fertilized egg attaches itself to our mother's womb. It draws nourishment; it grows, divides and multiplies and in nine months, we are born as a complete human being. By the time we are matured that single cell has replicated over one hundred trillion times. Each cell carries the same information as the first cell. We study a single cell.

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].

More than 20 years have passed since we sequenced the entire Human Genome that is we read the entire book of life of a human being, letter by letter, word by word and sentence by sentence and chapter by chapter (genes) all 46 volumes (Chromosomes) consisting of six billion four hundred million letter (nucleotides), the greatest book of life of a human on planet Earth.

Sequencing exome of a specific organ shows that out of 24,000 genes in our genome, our Brain requires 3,195 genes (to express) to function normally. This is the highest proportion of genes expressed in any part of the body. This study was conducted in identical and fraternal twins; around one-third to one-half of the individual variation in brain measures is due to genetic variations among us, and much of the rest is shaped by environmental factors ranging from education and diet to trauma and stress. While our brain requires 3,195 genes, our heart requires 1,195 genes and eye requires 545 genes. Using the second generation Nanopore sequencers and by comparing with the Reference Sequence, it would be easier, cheaper, and faster to identify mutations responsible for identifying any disorder in those organs. With the completion of the thousand genome project, we can compare the patient's mutated gene with the thousand Reference Sequence to identify the mutation with precision and accuracy. By sequencing the patient's genome and comparing it with the Reference Sequence, presents an additional advantage that can identify the mutations if it is autosomal, that it is occurred in the first 22 chromosomes (Every child born to an affected parent has a 50% chance of inheriting the genetic variant that causes the disease) or in germ-line cells that is if it is occurred in X and Y chromosomes. Mutations in the X and Y chromosomes are of great concerns because they could be passed on to the future generations.

Diagnosis, prevention, and the treatment is the ultimate goal of sequencing human genome. After diagnosing the mutations in the patient's genome, the next step is the prevention. If there is family history of mental illnesses, to avoid a disease in the next generation a couple can achieve conception by in vitro fertilization that is conception in the test-tube. By sequencing the embryo and selecting and implanting the very best embryo free from all mutations, and finally the treatment either by gene therapy or by drug therapy. Finally, even after successful diagnosis and prevention, treating mental disorders present the greatest challenge. Our brain is the most complex organ in the Universe, and it is protected by a fatty layer called the Blood Brain Barrier (BBB). Prevention is the responsibility of a pregnant mothers. Diagnosis and treatment of the mental disorders is our responsibility. A smoking pregnant mother should know that her growing fetus is exposed to hundreds of toxic chemicals and many of them are identified as cancer causing. Taking any drug legal or illegal would interfere with the growth and the development of the fetus. About one third of all genetic disorders show some neurological involvement, and many of these represent neurodegenerative diseases of infancy. Genetic or genetically influenced conditions rank among the leading causes of organically based mental retardations. Our challenge is to examine the molecular genetic mechanisms (genomic imprinting, triplet repeats, mitochondrial inheritance (gene mapping, enzymatic function, behavioral genetics, prenatal diagnosis, genetic counselling, epidemiologic studies, animal models, gene therapy and drug therapy.

During the old times, the following diseases were considered as the punishment from the Heaven for the misdeed of the patients' ancestors. Now, we know that these diseases have genetic origin. Not so long ago, all the horrendous diseases were untreatable. After sequencing the human genome, we have identified all the defected genes responsible for causing these diseases. Once the mutated genes are identified, we can design drugs to shut off those genes and cure the disease. We have moral obligation to take care of all those children who are already here. We can take precaution to prevent adding sick children in the highly competitive society where they are most like to be left behind. Over a dozen genetic disorders described below, the two most common genetic causes of intellectual disabilities are Down syndrome and Fragile X syndrome.

Down Syndrome

Down Syndrome is the leading genetic cause of mental retardation occurring in about one in one thousand births. Down syndrome (also known as trisomy 21) is an example of a condition caused by trisomy. People with Down syndrome typically have three copies of chromosome 21 in each cell, for a total of 47 chromosomes per cell resulting in the karyotype of 47, XXY. Double aneuploidy condition was first described in a patient with both Down and Klinefelter (48, XXY, +21) syndromes. This is also the most described double aneuploidy. Genome sequencing could easily identify the presence of an extra chromosome. Diagnosis makes it easier for a pregnant mother to terminate the pregnancy, but its treatment presents the greatest challenge. Chromosome-21 is one of the largest chromosomes in our genome. It is made of 50 million nucleotide base pairs and carries 337 genes. It is so complex; it is almost impossible to treat the disease. If it were monogenic or polygenic mutations responsible for causing the disease, by comparing with fetus's normal twin brother's genome as a Reference Sequence, we should be able to identify the specific mutations and develop treatment of the Down Syndrome. Some parents will do anything to save their Down baby but designing drugs to treat Down Syndrome is expensive and time consuming. The next generation scientists will explore all avenues including behavioral heterogeneity, critical genes on chromosome-21, Alzheimer-like pathology, gene dosage compensation, chromosomal nondisjunction, cellular mosaicism, animal models, epidemiology, and prenatal screening issues. If humans can survive with an extra chromosome, can we synthesize a chromosome by inserting useful genes such as high IQ, athletic abilities or singing abilities. Such studies are unlikely to be funded.

Fragile X Syndrome

Fragile X-Syndrome is the leading cause of heritable mental retardation, affecting about 1/1400 males and 1/2500 female children. Symptoms include a long and narrow face, large ears, a prominent jaw and forehead, unusually flexible fingers, and flat feet. The Fragile X syndrome gene is inherited and expressed in dominant pattern. It can be passed down from parents to children through conception. Since the gene for Fragile X is carried on the X chromosome; father carries (XY) and mother carries (XX), both parents have at least one X chromosome. Both parents can pass on the mutated gene to their children. Fragile X is never passed down from father to son. However, a father passes his only X chromosome to all his daughters, and therefore all daughters inherit his mutated gene. Fragile X syndrome is caused by a change to a gene on the X-chromosome called the FMR1 (Fragile X Messenger Ribonucleoprotein 1 or (FMR1) gene. This gene produces a protein that helps the brain to function normally. If this gene is changed or altered in any way, it cannot produce its normal protein, which can result in Fragile X syndrome. By comparing with the Reference Sequence, we can identify the mutation and design drugs to shut off that gene.

Klinefelter Syndrome

Klinefelter Syndrome is a sex chromosome disorder in boys that results from the presence of an extra X chromosome in cells. People typically have 46 chromosomes in each cell, two of which are the sex chromosomes. Females have two X chromosomes (46, XX), and males have one X and one Y chromosome (46, XY). Individuals with the 47, XXY karyotype carry millions of additional nucleotides. People with Klinefelter syndrome can experience breast growth, breast cancer, osteoporosis, infertility and learning difficulties. They usually show average or below-average intelligence performance, with IQs ranging from 80 to 109. They have Low sperm count or no sperm; Small testicles and penis; Low sex drive; Taller than average height; Weak bones; Decreased facial and body hair; Less muscular compared with other men; Enlarged breast tissue and Life expectancy for patients with Klinefelter syndrome is reduced by five to six years. Designing drugs to treat Klinefelter presents the greatest challenge because X-chromosome is made of 164 million nucleotide base pairs and carry 1,144 genes. By comparing with the Reference Sequence, we could identify defected genes responsible for causing the disorder. Designing drugs to shut off those genes would be expensive and time consuming. Pre-natal screening could help the pregnant mother to terminate the pregnancy. In vitro fertilization using the healthy embryo would protect the newborn. We described below a dozen preventable diseases from new born and to the old age diseases such as Alzheimer, Cardiovascular and Cancers.

The Following Additional Diseases Responsible for Intellectual Disabilities Associated with Genetic Mutations are Listed Below:

Alzheimer

Alzheimer disease is a degenerative disease of the brain that causes dementia, which is a gradual loss of memory, judgment, and ability to function. Alzheimer disease runs in the family. This disorder usually appears in people older than age 65, but less common forms of the disease also appear earlier in adulthood. Alzheimer does not have a single genetic cause. Instead, it can be influenced by multiple genes in combination with lifestyle and environmental factors. Consequently, a person may carry more than one gene or group of genes that can either increase or decrease the risk of Alzheimer. One well-known gene that influences Alzheimer's risk is the apolipoprotein E (APOE) gene. The APOE gene is involved in making a protein that helps carry cholesterol and other types of fat in the bloodstream. The presence of mutated proteins in their functions are thought to contribute to the development of Alzheimer. Patient inherits two APOE alleles, one from each biological parent. Scientists have identified more than 70 genetic regions associated with Alzheimer. They estimate that between 40-65% of people diagnosed with Alzheimer have the APOE-e4 gene. APOE-e4 is one of three common forms of the APOE gene; the others are APOE-e2 and APOE-e3. The function of each of the APOE alleles is described below: APOE ε2 may provide some protection against the disease. If Alzheimer occurs in a person with this allele, it usually develops later in life than it would in someone with the APOE ε4 gene. Roughly 5% to 10% of people have this allele. APOE ε3, which is the most common allele, and it is believed to have a neutral effect on the disease — neither decreasing nor increasing risk of Alzheimer. On the other hand, APOE ε4 increases risk for Alzheimer and is associated with an earlier age of disease onset in certain populations. About 15% to 25% of people have this allele, and 2% to 5% carry two copies. We all inherit a copy of some form of APOE gene from each parent. Of the genetic variants so far associated with Alzheimer, three rare single-gene variants, APP, PSEN1, and PSEN2 are known to cause the disease: They are Amyloid precursor protein (APP) located on chromosome-21 which is fairly long chromosome. It is made of 50 million nucleotide base pairs carrying 337 gene. Presenilin 1 (PSEN1) gene is located on chromosome-14 which is much longer. It is made of 109 million nucleotide base pairs carrying 1,173 genes. and Presenilin 2 (PSEN2) located on chromosome-1 which is the longest chromosome in the human genome. It is made of 263 million nucleotide pairs carrying 2,610 genes. There is no cure to treat Alzheimer at this time. After sequencing the entire human genome, we can now sequence the specific genes responsible for causing Alzheimer and compare its sequence with the Reference Sequence to identify specific nucleotide mutations. Furthermore, we can compare mutations sequence with the sequence of the thousand genome project to identify specific nucleotides with precision and accuracy. Once we identify the mutated nucleotide, our greatest challenge is to design drugs to specifically shut off mutated genes without harming the normal genes. Our work on AZQ (US Patent 4,233,215) described below show how to design drugs to shut off a gene.

Epilepsy

Epilepsy is a genetic defect caused by an imbalance of nerve-signaling chemicals called neurotransmitters, tumors, strokes, and brain damage from illness or injury, or some combination of these. Who is most likely to get epilepsy? It can start at any age, but usually starts either in childhood or in people over 60. Experts believe that, in many cases, genetic predisposition combined with environmental conditions lead to epilepsy. About 30 to 40 percent of epilepsy is caused by genetic predisposition. First-degree relatives of people with inherited epilepsy have a two- to four-fold increased risk for epilepsy. Some studies[3] have found that epilepsy is more likely to be passed down from the mother than the father. Genetic Epilepsy includes, Angelman syndrome, CDKL5, PCDH19, Ring chromosome 20, SCN8A related, SLC2A1 (Glut1 Deficiency Syndrome, TBCK-related ID, Rett-MECP2. People with epilepsy may be concerned about passing on their seizure disorder to their children. However, most children born to parents who have epilepsy do not develop seizures. If the father does not have epilepsy and the mother has epilepsy, the chances are less than five percent. If genome sequencing identifies mutations on any of the above specific genes responsible for causing Epilepsy, it is possible that the next generation of scientists could design drugs to shut of those genes.

Duchenne Muscular Dystrophy

Duchenne muscular dystrophy is a rare, genetic condition that is characterized by progressive muscle damage and weakness. Muscular dystrophy (MD) refers to a group of more than 30 genetic diseases. This rare disease is caused by a genetic mutation that prevents the body from producing a protein called dystrophin. This protein is located primarily in skeletal and cardiac muscle, where it helps stabilize and protect muscle fibers. Dystrophin acts like a shock absorber when muscles contract. Without dystrophin, muscles become more and more damaged and weakened. They may also lose the ability to repair themselves after an injury. Over time, children with Duchenne will develop problems walking and breathing, and eventually, the heart and the muscles that help them breathe will stop working. Duchenne is an irreversible, progressive disease. While there have been many advancements in the management of Duchenne, there is no cure at present. Duchenne primarily affects males, with 1 in 3,500 to 5,000 boys born worldwide having Duchenne. In rare cases, it can also affect females. Duchenne muscular dystrophy is an X-linked recessive disorder that affects 1 in 3,500 males and is caused by mutations in the dystrophin gene. It is largest gene in the human genome, encompassing 2.6 million base pairs of DNAs and containing 79 exons. By sequencing the patient's whole genome and comparing with Reference Sequence, we can identify Mutations responsible for causing the disease. Once the specific mutated nucleotide is identified, we can design drugs to shut off its expression and find a cure.

Schizophrenia

Schizophrenia is a mental health disorder that affects millions of people worldwide. It is a chronic condition that interferes with a person's ability to think, feel, and behave clearly. People with schizophrenia often experience a range of symptoms, including delusions, hallucinations, disordered thinking, and cognitive difficulties. While the causes of schizophrenia are not completely understood, researchers believe that a combination of genetic, environmental, and biological factors may play a role in its development. Schizophrenia tends to run in families, but no single gene is thought to be responsible. If both your parents have it, you have a 40% chance of getting it. Mutations such as deletion or duplication on several chromosomes affecting multiple genes are thought to increase schizophrenia risk. A small deletion (mi-

crodeletion) in a region of chromosome-22 may be involved in a small percentage of cases of schizophrenia. Chromosome-22 is a very large chromosome. It is made of 56 million nucleotide base pairs and carries 701 genes. By sequencing the patient's chromosome-22 and comparing with the Reference Sequence, if we confirm that suspect region on Chromosome-22 called 22q11is involved, then we can design drugs to shut of that gene to prevent its expression. Several potential genes have received a good deal of attention. These include neuregulin (NRG-1, 8p21-p12), dysbindin (DTNPB1,6p22.3), G72 (13q34)/D-amino acid oxidase (DAAO, 12q24), proline dehydrogenase (PRODH2, 22q11.21), catechol-O-methyltransferase (COMT, 22q11.21) and regulator of G protein signaling (RGS-4,1q21–q22). The role of functional candidate genes, 5HT2A and dopamine D3 receptor (DRD3) have also been identified. Presently, Antipsychotic drugs are used to treat schizophrenia and related disorders without knowing how they work.

Huntingdon Chorea

Huntington's disease (HD) is an autosomal dominant disorder, and it is an inherited disorder that causes nerve cells (neurons) in parts of the brain to gradually break down and die. It is passed from parent to child through a mutation (a defect) in a particular gene. When a parent has HD, each child has a 50 percent chance of inheriting the copy of chromosome 4 that carries the HD mutation. Huntington's disease is caused by an inherited difference in a single gene. DNA contains a long string of four nucleotides made of A/T and G/C. A chromosome is a long string of DNA made of millions of nucleotides. A gene is a unit of inheritance and its codes for a protein. It is a piece DNA located on chromosomes. A gene on a chromosome is identified by a START Codon (AUG codon codes for amino acid Methionine). The codon AUG initiate the synthesis of DNA and the DNA synthesis stops when one of the three STOP codons appears: UAG, UGG, UGA. For example, Chromosome-4 is made of 203 million nucleotides, and it carries 1,024 genes. A mutation on Chromosome-4 is responsible for causing Huntingdon disease. This defect is "dominant," meaning that anyone who inherits it from a parent with Huntington's will eventually develop the disease. In autosomal dominant disorder, which means that a person needs only one copy of the nontypical gene to develop the disorder. each child of a parent with HD has a 50 percent chance of inheriting the HD gene. A child who does not inherit the HD gene will not develop the disease, and generally, they cannot pass it on to their children or other future generations. HD is caused by a mutation in the gene for a protein called huntingtin. The defect causes the building blocks of multiple codons CAG (cytosine, adenine, and guanine) which repeats many more times than they normally do. Most normal people have fewer than 27 CAG repeats in their HD gene, so they are not at risk for the disease. People who have CAG repeats in the middle range (27 to 35) are not likely to develop the disease, but they could still pass it on this mutation to future generations. Patients with HD who may have 36 or more CAG repeats come down with HD. Huntington's disease is a progressive brain disorder caused by a single defective gene on chromosome 4 — one of the 23 human chromosomes that carry a person's entire genetic code. It is believed to be caused by a single-nucleotide substitution in TITF1, located on chromosome-4. A wide spectrum of mutations has been reported, drawing a potential association between amount of subsequently deleted nucleotides to severity of symptoms. If confirmed by comparing the sequence of this gene with Reference Sequence, and with the thousand genome project, we can design drugs to treat HD and reject the old idea that you cannot do anything to prevent HD, if you inherit the gene.

Parkinson Disease

Parkinson's disease is a progressive brain disorder that is caused by degeneration of nerve cells in the part of the brain called the substantia nigra, which controls body movement. Due to certain mutations, these nerve cells die or become impaired, losing the ability to produce an important chemical called dopamine. Symptoms of Parkinson's disease (PD) includes unintended or uncontrollable movements, such as shaking, stiffness, and difficulty with balance and coordination. Symptoms usually begin gradually and worsen over time. As the disease progresses, affected people may have difficulty walking and talking. It was believed that mutations in certain genes are either inherited or passed down from generation to generation. Now, we confirm that to date, two genes responsible for familial Parkinson's disease have been identified: one is the alpha-synuclein gene located in the long arm of chromosome 4, and the other is the parkin gene located in the long arm of chromosome 6. Gene mutations for other diseases on chromosome 4 include neurological and neurodegenerative disorders such as Parkinson's disease, Huntington's disease, and narcolepsy. Chromosome-4 and chromosome-6 are some of the largest chromosomes in our genome. Chromosome-4 is made of 203 million nucleotide base pairs, and it carries 1,024 genes. A mutation on Chromosome-4 is responsible for causing both HD and PD. Chromosome-6 is also one of the largest chromosomes which is made of 183 million nucleotide base pairs carrying 1,396 genes. Mutation in the UCHL1 gene causes autosomal dominant Parkinson's disease. Mutations in the glucocerebrosidase beta (GBA) gene are the most common of the currently known PD genetic mutations. GBA mutations increase a person's risk of Parkinson's, but less so than mutations in SNCA or LRRK2. Familial cases of Parkinson disease can be caused by mutations in the LRRK2, PARK7, PINK1, PRKN, or SNCA gene. Sequencing the genome of PD patient and comparing with the Reference Sequence, will identify mutated genes with certainty, but the precision and accuracy can be obtained by comparing with the thousand genome project. Once mutated genes are identified, we can design drugs to treat PD by shutting off these genes.

Hemophilia

Hemophilia is a genetic disease passed down from parents to children. It is a medical condition in which patients lose the ability to clot the blood causing them to bleed severely even from a slight injury. When blood can't clot properly, excessive bleeding (external and internal) occurs after any injury or damage to cells resulting from many large or deep bruises, joint pain swelling, unexplained bleeding, and blood in urine or stool. Hemophilia is caused by a mutation in one of the genes on X-chromosome, that codes for making the proteins needed to form a blood clot. This mutation can prevent the clotting protein either from working properly or to be missing altogether. There are three kinds of Hemophilia, and they are Hemophilia A: This is the most common type of hemophilia due to lack of factor VIII. Hemophilia B happens when you don't have enough clotting factor 9 (factor IX.) and Hemophilia C happens due to lack of factor 11 (factor XI). Mutations in the F8 gene cause hemophilia A, while variants in the F9 gene cause hemophilia B. The F8 gene codes for making a protein called coagulation factor VIII. A related protein, coag-

ulation factor IX, is produced from the F9 gene. By sequencing X and Y-chromosomes, these defected genes can be identified before conception and save children from unnecessary suffering. If mutations on X-chromosome is confirmed, conception by in vitro fertilization is the best course. Children are loving union of parents. A mother's egg receives a father's sperm, and they are conceived. A mother produces a single mature egg each month if not used it is washed down. The nucleus of the Egg always carries an X-chromosome which is a large chromosome made of 164 million nucleotide base pairs consisting of 1,144 genes. On the other hand, a father produces millions of sperms each time. Not all sperms are healthy, some mature, some immature and others are broken. A healthy baby is born when a healthy egg is fertilized by a healthy sperm. While half of the sperm carries X-chromosome, another half carries Y-Chromosome. Compared to X, the Y-Chromosome is smaller, consisting of 59 million nucleotide base pairs carrying 231 gene. Mother always provides an X-chromosome, but during conception, if her egg receives a sperm carrying X-chromosome, the fertilized egg results in an XX embryo giving birth to a girl. On the other hand, if her egg receives a sperm carrying a Y-chromosome the fertilized egg will carry an XY embryo giving birth to a boy. Hemophilia is a sex-linked recessive disorder. The abnormal gene responsible for causing hemophilia is carried on the X chromosome. A male can have hemophilia if he inherits an affected gene on the X-chromosome (an X chromosome with a mutation in the gene that causes hemophilia) from his mother. The mother is the one who passes the hemophilia gene to her fetus. Female child carries two X-chromosome. if mutation occurs on one X-chromosome, the female child will not be affected, because it is protected by the other healthy X-chromosome. The female child only becomes a carrier. On the other hand, if a male child receives the defected X-chromosome, it has no protection for Hemophilia, it will exhibit the condition. In male child (who have only one X chromosome), one altered copy of the gene in each cell is sufficient to cause Hemophilia. Hemophilia almost always is inherited and is passed down from a parent to a child. Both hemophilia A and B are affected in the same way, because both the genes for factor VIII and factor IX are located on the X chromosome. If sequence of the mother's X-chromosome identifies the presence of mutated genes and confirmed by comparing with the Reference Sequence and the thousand genome project, we can design drugs to shut off those genes.

Tay-Sacks Disease (TSD)

Tay-Sachs disease is a rare, neurodegenerative disorder in which deficiency of an enzyme (hexosaminidase A) results in excessive accumulation of certain fats (lipids) known as gangliosides in the brain and nerve cells. This abnormal accumulation of gangliosides leads to progressive dysfunction of the central nervous system. Tay-Sachs disease is categorized as a lysosomal storage disease. Lysosomes are the major digestive units in cells. Enzymes within lysosomes break down or "digest" nutrients, including certain complex carbohydrates and fats (like glycosphingolipids). When one of these lysosomal enzymes (such as hexosaminidase A) is missing or mutated and ineffective, glycosphingolipids start to build up in the lysosome. If there is too much accumulation of lipids in the lysosome, the cells in the nervous system degenerate and die, triggering an inflammatory response that amplifies damage in surrounding tissues.

Tay-Sachs is an autosomal recessive disease caused by mutations in both alleles of a gene (HEXA) on chromosome 15. HEXA codes for the alpha subunit of the enzyme β-hexosaminidase A. This enzyme is found in lysosomes, organelles that break down large molecules for recycling by the cell. Tay-Sachs disease is caused by a frameshift mutation on chromosome 15. A person with this disease lacks the enzyme hexosaminidase A. This enzyme is needed to break down gangliosides, a fatty substance that is formed in nerve cells and in the brain. Chromosome-15 is made of 106 million nucleotide base pairs consisting of 906 genes. A frameshift mutation in a patient with Tay-Sachs disease causes premature termination and defective intracellular transport of the alpha-subunit of beta-hexosaminidase. A four base pair insertion in exon 11 (1278insTATC) results in an altered reading frame for the HEXA gene. As the disease progresses, children with Tay-Sachs disease experience involuntary muscle twitches (myoclonic jerks), seizures, difficulty swallowing (dysphagia), vision and hearing loss, and intellectual disability. An eye abnormality called a cherry-red spot, which is identified by eye examination, is characteristic of this disorder. Children with infantile Tay-Sachs disease usually live only into early childhood. By comparing with the sequence of the mutated genes responsible for the production of enzyme hexosaminidase A and comparing with the sequence of the thousand genome project sequence if we confirm the exact location of the mutation with precision and accuracy, then we can design drugs to shut off that gene.

Cystic Fibrosis

Cystic fibrosis (CF) is an inherited life-threatening disorder that damages the lungs and digestive system. It affects the cells that produce mucus, sweat, and digestive fluids. CF causes these fluids to become thick and sticky. These fluids plug up tubes, ducts, and passageways. CF is caused by mutations in a gene called the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. The CFTR gene provides instructions to make the CFTR protein. The gene responsible for causing CF is found on Chromosome-7. There are many mutations (defected genes) that have been shown to cause CF disease. People with only one copy of the defective CF gene are called carriers. People with cystic fibrosis have two non-working copies (alleles) of the gene, and so they make little or no CFTR protein. A child can inherit CF only if both parents carry a CF gene (that is, each parent either has CF or is a carrier) and both parents pass the CF gene to their child. This can happen if the parents are "carriers" of the faulty gene. The mutated gene is identified on the long arm (q) of Chromosome-7 (7q31. 2). This gene is very large and complex. Chromosome-7 is itself a long chromosome and is made of 171 million nucleotide base pairs consisting of 1,378 genes. More than 1,800 different mutations in this gene have been found, but there are about 30 genes that are common. The most common gene mutation is called deltaF508. There's currently no cure for CF, but a number of treatments are available to help control the symptoms to prevent complications, and make the condition easier to live with. One possible treatment includes the use of antibiotics to prevent and treat chest infections. We need to develop new drugs to treat CF. Once the gene is identified by comparing with the Reference Sequence and confirmed by comparing with the sequence of the Thousand Genome Project, we could start designing drugs to shut off those genes.

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

After Sequencing the Human Genome, Our Search for Unknown Diseases has Come to a Closure

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

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

Gene Therapy

The first step is to cut the human genome with specific enzymes (prepare a Restriction Site Map) at the specific sites using restriction enzymes (molecular scissors such as EcoR1) first accomplished by El Salvador Luria, Max Delbruck, and Hamilton Smith. The fragment of human DNA (a single gene) if not protected will be destroyed by antibody. A naked gene is a piece of DNA (which has a start codon AUG and after a few thousand nucleotide (codons) end at one of the three stop codons UAG, UGA or UGG if not protected by recombinant technology (making a hybrid) that is by recombining with the DNA of Virus, or Plasmids, or Chloroplasts (for plants) which serves as Vectors. If not protected it will be destroyed by enzymes. One can store the fragments or genes in the Vectors once the human DNA fragment is stabilized in Vectors by recombinant technology; we can not only purify this fragment (genes), but also, we can make millions of copies (clone) of this fragment of DNA by transferring into the host cells such as Bacteria, mammalian cells or Yeast cell which autonomously replicates to produce library of genes. Each Library contains millions of copies of identical genes that produce the same protein. Before the genetic revolution, Insulin is extracted from pancreas of the slaughtered animals which is used to treat old diseases such as diabetes; a tiny fragment of impurity could set anaphylactic shock and kill the patients. Now, large scale highly pure human Insulin produced by Genetic Engineering firm named Genentech is used to treat 300 million diabetic patients worldwide without the loss of a single life. Other products of Genomic Medicine such as Growth hormones and hormone proteins to treat Hemophilia by factor VIII protein are being developed as genomic medicines by recombinant technology. Attempts are being made to design drugs to attack cancer cells on all three levels that is DNA, RNA and Protein. Herceptin, a novel class of drug, has been successful in attacking protein. Craig Milo has designed double stranded RNA to shut off gene and prevents its translation into protein. One of the greatest challenges in designing drugs is to attack the DNA to shut off a gene. It was successfully carried out by Ross using highly toxic Nitrogen Mustard.

Drug Therapy

Gene Therapy cannot be applied to treat diseases with multiple genetic defects such as cancers or heart diseases. Drug Therapy could be used to develop novel treatments.

How to Design Drugs to Shut off a Gene?

Historical Background for Using Nitrogen Mustard for Treating Cancer

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

Ross' Rationale for using War Chemicals to Treat Cancers

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

could attach the Nitrogen Mustard group to the dye and attack the cancer genes in any one those 220 tissues.

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

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

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

Synthesis of Nitrogen Mustard as Anti-Cancer Drugs

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

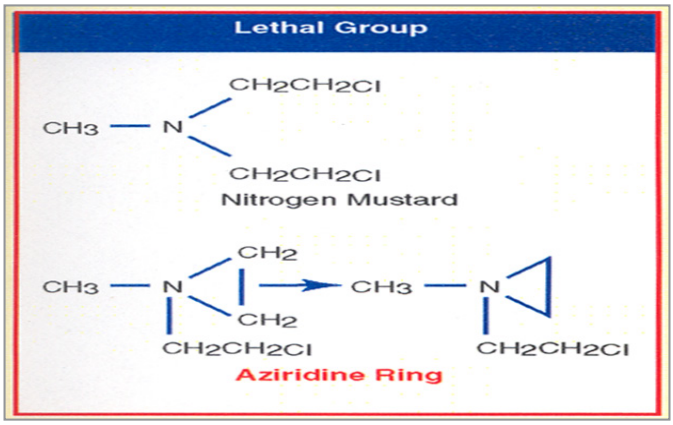
As I said above, I had made several dozens of analogs of Nitrogen Mustards for Professor Ross. I will describe how to make the Nitrogen Mustard by using Haber's crudest method. Haber reacted Methylamine with Ethylene oxide to make 2-bis dihydroxy ethyl methyl amine. It was chlorinated by heating with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

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

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

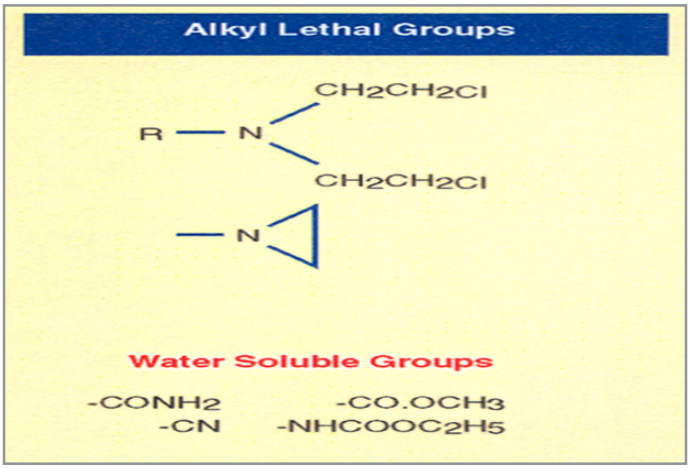
A radiolabel study to understand the mechanism of action of Nitrogen Mustard showed that cross-linking of DNA occurred in two steps. The first step is involved in the formation of a three-member aziridine intermediate which remains stable and inactive in the neutral media (acts as a pro-drug). The second arm of the Nitrogen Mustard generates a highly reactive carbonium ion by enzyme which attacks the first arm of the double stranded DNA. The second arm is attacked, as the cancer cells grow; they use Glucose as a source of energy. Glucose is broken down the Lactic Acid. In the presence of acid, the Aziridine ring become activated by generating the carbonium ion which attacks the second arm of the DNA resulting in the cross-linking.

This study result showed that cross-linking both strands of DNA is not necessary to shut off a gene, only binding to a single strand of DNA by aziridine could also shut off a gene with half the toxicity. To attack a single strand of DNA, aziridine analog are separately synthesized. As a part of my doctoral thesis, I was assigned a different path. Instead of cross-linking DNA strands, I am to design drugs to attack only one strand of DNA. The following chart describes the formation of Aziridine ring intermediate.



DNA Binding Aziridine Group

This study showed that to attack a single strand of DNA, we must synthesize Aziridine in the Lab by using ethyl amino methyl sulphonate in sodium hydroxide. Pure Aziridine was distilled off. Synthesis of Aziridine analogs will give two advantages over Nitrogen Mustard: first, instead of cross-linking, Aziridine binds to one strand of DNA, reducing its toxicity of the double stranded Nitrogen Mustard by half. Second, it gives selectivity, the Aziridine ring serves as a prodrug. Its ring opens only in the acidic medium. Once the active ingredient Aziridine was determined to attack DNA, the next question was what drug delivery method should be used to deliver Aziridine at the tumor site.



The above Structures are Nitrogen Mustard (2-Bischloroethyl Methyl Amine) and Aziridine.

DNA Binding Lethal Groups

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

As a part of my doctoral thesis, I was assigned a different path. Instead of cross-linking both strands of DNA by Nitrogen Mustard, I am to design drugs to attack only one strand of DNA by making Aziridine analogues. We decided to use Aziridine moiety (as an intermediate of Nitrogen Mustard) that would be an excellent active component to shut off a gene by binding to a single strand of DNA. To deliver Aziridine to the target site which is the N-7 Guanine of DNA, we decided to use Dinitrophenyl (DNP) moiety as a drug delivery agent. DNP is a dye which colors the tissues of the experimental animal tumor such as Walker Carcinoma 256 in Rats.

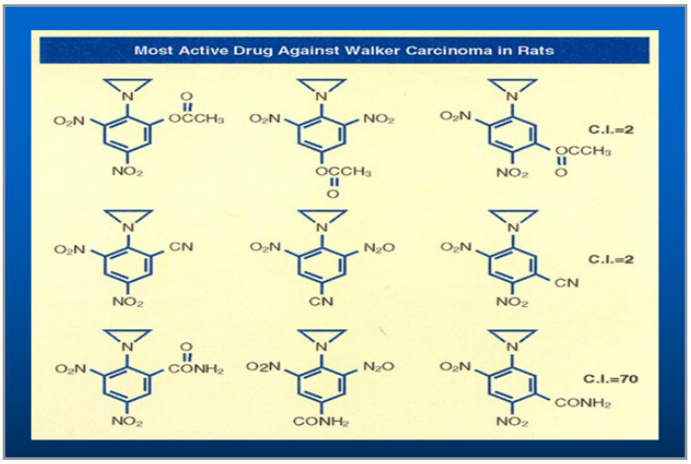
It is well known that analogs of DNP such as Dinitrophenol disrupts the Oxidative Phosphorylation of the ATP (Adenosine Triphosphate) which provides energy to perform all our body functions. To provide energy to our body function, the high energy phosphate bond in ATP is broken down to ADP (Adenosine Diphosphate) which is further broken down to AMP (Adenosine Mono Phosphate), the enzyme Phosphokinase put the inorganic phosphate group back on the AMP giving back the ATP. This cyclic process of Oxidative Phosphorylation is prevented by Dinitrophenol. As a part of my doctoral thesis, I decided to use Dinitrophenol as drug delivery method for the active ingredient aziridine. The analog of DNP such as Aziridine Dinitrophenol could also serves as a dye which stains Walker Carcinoma 256, a solid and most aggressive tumor in Rat. The first compound I made by attaching the C-14 radiolabeled Aziridine to the DNP dye. The Dinitrophenyl Aziridine was synthesized using Dinitrochlorobenzene with C-14 radiolabeled Aziridine in the presence of Triethyl amine which removes the Hydrochloric Acid produced during the reaction. When the compound Dinitrophenyl Aziridine was tested against the implanted experimental animal tumor, the Walker Carcinoma 256 in Rats, it showed a TI (Therapeutic Index) of ten. The TI of ten was like most of the analogs of Nitrogen Mustard. Since this Aziridine analog was not superior to Nitrogen Mustard, it was dismissed as unimportant.

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

Structure Activity Relationship

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

An additional substituent in the Dinitrophenyl Aziridine could give three isomers, Ortho, Meta, and Para substituent. Here confirmational chemistry plays an important role in drug delivery method. Ortho substituent always give inactive drug. Model building showed that because of the steric hinderance, Aziridine could not bind to DNA shutting off the genes. On the other hand, Meta and Para substituents offer no steric hindrance and drug could be delivered to DNA. When injected in Rat, because of the high solubility, most of the drugs was pass down through urine and extracted the drug from Rat urine by chloroform, The following chart showed that I synthesized all nine C-14 radiolabeled analogs of 2,4-Dinitrophenyl aziridines and tested them against implanted Walker Carcinoma 256 in Rats.



Derivatization of Dinitro phenyl Benzamide based on Partition Coefficient

The Most Water-Soluble Substituent

The first three compounds on top line of the above chart carry all three isomer of the most water-soluble Ethyl Ester group attached to 2,4-Dinitropehny aziridine. The compound in vivo is hydrolyzed Ethyl Ester to produce most water-soluble carboxylic group. Since it is the most water-soluble substituent, within 24 hours of injection in Rats, the entire radioactive compound was passed down from in the Rat urine and it can be extracted by Chloroform. Since the Ortho position was not available for DNA binding, it showed no biological activity, but the third compound in which Ortho position was free to bind to DNA showed some anti-tumor activity in Rats.

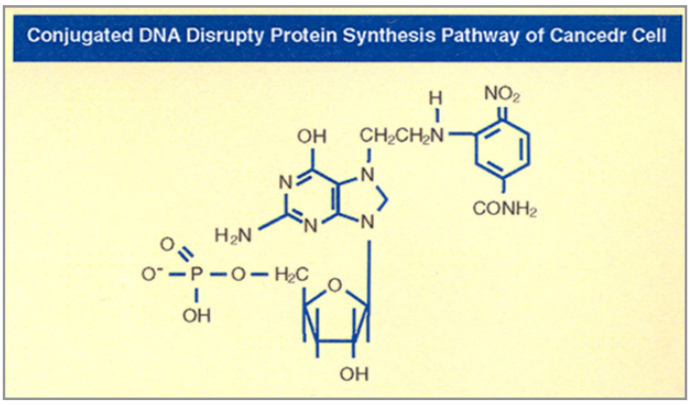
The Least Water-Soluble Substituent

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

The Moderately Soluble Amido-Substituent

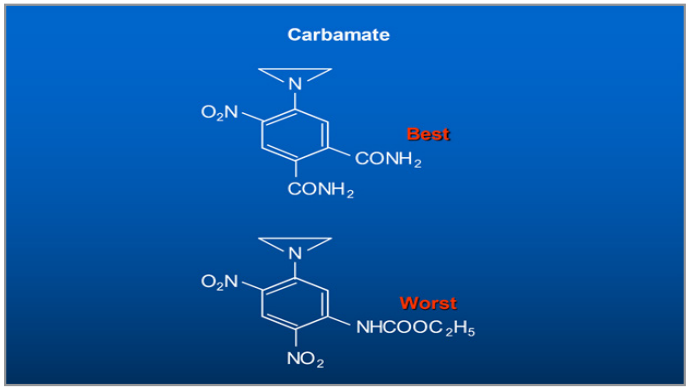
The last line of the above chart showed that the first two Amido groups were sterically hindered and did not bind to DNA and showed no biological activity, but the last compound presents the perfect drug delivery method. The entire drug was delivered from the injection site to the tumor site. The drug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

As I said above, Nitrogen Mustards are highly toxic because they have neither specificity nor selectivity. They attack all dividing cells whether they are normal or abnormal. On the other hand, the analogs of Aziridines and Carbamates serve as prodrug and remain inactive in the basic and neutral media. They become activated only in the presence of acid produced by growing cancer cells. Aziridine attacks DNA in acidic medium, particularly the N-7 Guanine. The dye Dinitro benzamide has great affinity for Walker Tumor. The Aziridine Dinitro benzamide (CB1954) has the highest toxicity to Walker Tumor cells ever recorded. As the tumor grows, it uses Glucose as a source of energy. Glucose is broken down to Lactic Acid. It is the acid which activates the Aziridine ring. The ring opens to generate a carbonium ion which attacks the most negatively charged N-7 Guanine of DNA (as shown below) shutting off the Walker Carcinoma gene in Rat. The following conjugate structure show how CB1954 binds to a single stranded of DNA shutting off the gene.



Conjugated DNA Disrupting Protein Synthesis Pathway of Cancer Cell

For the discovery of CB1954, The University of London, honored with the Institute of Cancer Research (ICR) post-doctoral fellowship award to synthesize more analogs of CB1954. To improve drug delivery method, over the years, I made over a hundred additional analogs of Dinitro phenyl aziridines. To increase the toxicity of CB1954 to Walker Carcinoma, I made additional 20 analogs as a postdoctoral fellow. When I attached one more Carbonium ion generating moiety, the Carbamate moiety to the Aziridine Dinitrobenzene, the compound Aziridine Dinitro benzamide Carbamate was so toxic that its Therapeutic Index could not be measured. We stop the work. Further work in London University was discontinued for safety reason.



The Best and the Worst Dinitro Phenyl Aziridine Analogs

Although Aziridine Carbamate is extremely toxic, it is also very useful in testing the sensitivity of tumors in Tumor Bank. Over

the years, some tumors in the tumor bank could become resistant. If a tumor culture survives in a petri dish by adding a solution of Aziridine Dinitrobenzene Carbamate, it means that this tumor has become resistant over the years and must be replaced by new sensitive tumor cells.

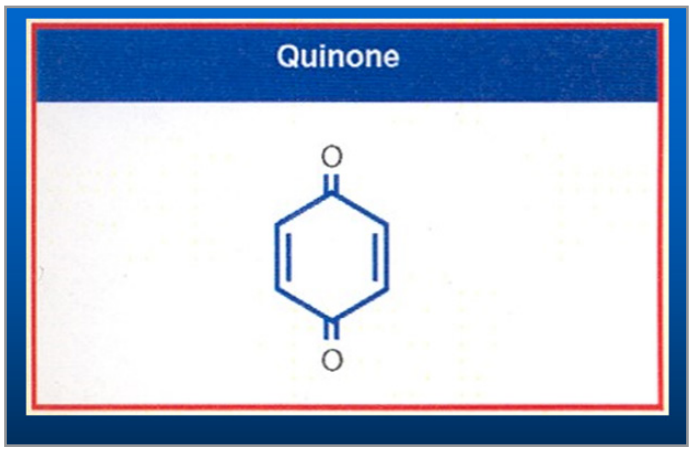
As a part of the inter-government agreement between UK and USA, all novel drugs developed in England were sent to the National Cancer Institute (NCI) in America for further screening. To translate animal work to human, I was invited to continue my work on the highly toxic Aziridine/Carbamate combination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

In developing drugs for treatments, we poison bad DNA selectively. All poisons are a class of chemicals that attacks all DNA good and bad alike. Chemicals that cause cancer, at a safe level, can also cure cancer. Science teaches us to selectively attack bad sets of DNAs without harming the good sets of DNAs. Poisons are injurious to living creatures. There is a small class of chemical, when exposed to humans, disrupt the function of DNAs, and make normal cells abnormal and they are called cancer causing chemicals or carcinogens. I must confess, we still use surgery to cut off a cancerous breast; we still burn cancer cells by radiations; and we still poison cancer cells by chemicals. The largest killer of women is breast cancer. After all the treatment, the remaining cancer cells return as metastatic cells and kill breast cancer patients in three years. A decade from now, these methods could be considered as brutal and savage, but today that is all we have. We hope to develop new treatment for Breast Cancer. Hopes means never ever to give up.

Glioblastoma (GBM) is a primary type of brain cancer which originates in the brain, rather than traveling to the brain from other parts of the body, such as the lungs or breasts. GBM is also called glioblastoma multiforme which is the most common type of primary brain cancer in adult humans. Attaching Nitrogen Mustard group to a carrier dye will produce highly toxic compound which will have neither specificity nor selectivity. Such a compound will attack all dividing cells whether they are normal or abnormal. On the other hand, the analogs of Aziridines and Carbamates serves as prodrugs that is they remain inactive in the basic and neutral media. They become activated only in the presence of acid produced by cancer cells.

Designing Drugs to Treat Glioblastoma, the Human Brain Cancers

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



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

With the Quinone ring, I could introduce two Aziridine rings and two Carbamate moieties and could create havoc for Glioblastoma. Within three years, I made 45 analogs of Quinone. One of the Quinone carries two aziridines and two carbamate moieties which was highly toxic to Glioblastoma. The tumor stops growing and started shrinking. I named the Di-aziridine Dicarbamate Quinone, AZQ. My major concern was how toxic this compound would be to the normal brain cells. Fortunately, brain cells do not divide, only cancer cells divide. AZQ acts as a Prodrug. A Prodrug is compound carrying a chemical by masking group that renders it inactive and nontoxic. Once the prodrug reaches a treatment site in the body, removing the mask frees the active drug to go only where it is needed, which helps avoid systemic side effects. Aziridine and Carbamate show selectivity. As I said above, to grow rapidly, cancer cells use Glucose as a source of energy. Glucose is broken down to produce Lactic acid. It is the acid which activates the prodrug aziridine and carbamate moieties generating Carbonium ions attacking Glioblastoma which stop growing and start shrinking.

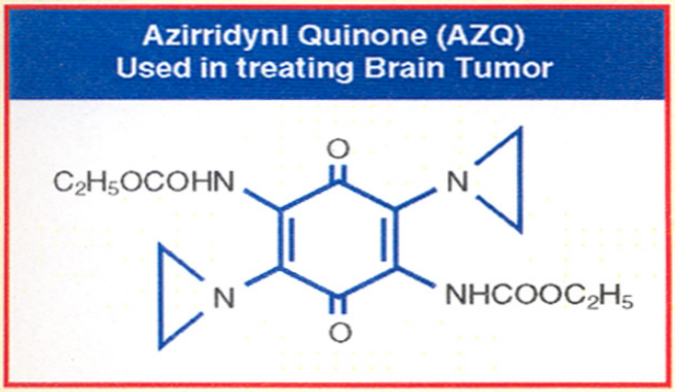
My drug AZQ is successful in treating experimental brain tumor because I rationally designed to attacks dividing DNA. Radio labeled studies showed that AZQ bind to the cancer cells DNA and destroy brain tumor and normal brain cells are not affected at all. AZQ is a new generation of drugs. Not so long ago, brain cancers mean death. Now, we have changed it from certain death to certain survival. The immunologists in our laboratories are developing new treatment technique by making radio labeled antigens to attack remaining cancer cells without harming normal cells.

We have cured many forms of cancer. We have eliminated childhood leukemia, Hodgkin disease, testicular cancer and now AZQ type compounds which are being developed rationally. While most anti-cancer drugs such as Adriamycin, Mitomycin C, Bleomycin etc., in the market are selected after a random trial of thousands of chemicals by NCI, AZQ is rationally designed for attacking the DNA of cancer cells in the brain with-

out harming the normal cells. We are testing combinations of these drugs to treat a variety of experimental cancers in animals [17, 18].

Single Strand DNA Binding Aziridines

I decided to use Quinone moiety as a carrier for Aziridine rings to attack Glioblastomas. By introducing an additional Carbamate moiety, I could increase its toxicity several folds. I planned to use this rational to translate animal work to human by introducing multiple Aziridine and Carbamate moieties to the Quinone to test against Glioblastomas in humans. Over the years, I made dozens of analogs of Aziridine Quinone. By attaching two Aziridines and two Carbamate moieties to Quinone, I synthesized the most useful compound, Diaziridine Dicarbamate Quinone, I named this novel compound AZQ. Over three-year period, I made 45 analogs of AZQ. They were all considered valuable enough to be patented by the US Government (US Patent 4,233,215). By treating brain cancer with AZQ, we observed that Glioblastoma tumor not only stops growing, but it also starts shrinking. I could take care of at least one form of deadliest old age cancers, Glioblastomas. Literature search showed that AZQ is extensively studied as a pure drug and in combination with other anti-cancer drugs.



U.S. Patent 4,146,622

Single Strand DNA Binding Aziridine and Carbamate

As I said above, Glioblastomas, the brain cancers, is a solid and aggressive tumor and is caused by mutations on several sites in chromosomal DNA. Deleterious genetic mutations are the result of damaging to DNA nucleotides by exposure to radiations, chemical and environmental pollution, viral infections, or genetic inheritance. The other factors responsible for causing DNA mutations are due to the fast rate of replication of DNA. For example, the bacteria E-coli grows so rapidly that within 24 hours, a single cell on a petri dish containing nutrients forms an entire colony of millions when incubated on the Agar Gel. Mistakes occur in DNA during rapidly replication such as Insertion of a piece of DNA, Deletion, Inversion, Trans location, Multiple Copying, Homologous Recombination etc. When an additional piece of nucleotide is attached to a DNA string, it is called Insertion, or a piece of DNA is removed from the DNA string; it is called Deletion or structural Inversion of DNA is also responsible for mutations. Since the gene codes for Proteins, Insertion and Deletion on DNA have catastrophic effects on protein synthesis. With the Quinone ring as a carrier across BBB, I could introduce different combinations of Aziridine rings and Carbamate moieties to Quinine and could create havoc for Glioblastomas. My major concern was how toxic this compound would be to the human brain cells. Fortunately, brain cells do not divide, only cancer cells divide. Attempting to find the site of mutations on Glioblastomas represent the greatest challenge. In Glioblastomas, three major changes occur on Chromosomes (C-7, C-9 & C-10) and two minor changes occur on Chromosomes (C-1 & C-19). These mutations are responsible for causing brain cancers in humans. Let us examine the effect on each chromosome. In a normal human cell, Chromosome-7 which is made of 171 million nucleotide base pairs, and it carries 1,378 genes. When Insertion occurs on Chromosome-7. Ninety-seven percent of Glioblastoma patients are affected by this mutation. On the other hand, a different mutation occurs on Chromosome-9 which is made of 145 million nucleotide base pairs, and it carries 1,076 genes. A major Deletion of a piece of DNA occurs on Chromosome-9 which results in eighty- three percent patients who are affected by this mutation. A minor Deletion of DNA also occurs on Chromosome-10 which is made of 144 million base pairs, and it carries 923 genes. Although it is a minor deletion of a piece of DNA and yet it contributes to ninety-one percent patients with Glioblastoma. To a lesser extent, small mutation occurs on Chromosome-1 (the largest Chromosome in our Genome). It is made of 263 million nucleotide base pairs and carries 2,610 genes) and Chromosome-19 (it is made of 67 million base pairs and carries 1,592 genes) is also implicated in some forms of Glioblastomas.

All known Glioblastomas causing genes are located on five different chromosomes and carries a total of 9,579 genes. It appears impossible to design drugs to treat Glioblastomas since we do not know which nucleotide on which gene and on which chromosome is responsible for causing the disease. It becomes possible by using C-14 radiolabeled Aziridines, we can confirm the binding site of a nucleotide on a specific gene and on a specific chromosome. By comparing with the mega sequencing genome project, we can further confirm the sites of mutations.

With the completion of 1,000 Human Genome Project, it becomes easier. By simply comparing the patient's genome with the sequencing of 1000-genomes, letter by letter, word by word and sentence by sentence, we could identify the differences called the variants with precision and accuracy, the exact variants, or mutations responsible for causing the disease. Once the diagnosis is confirmed, the next step is how to treat the disease. As I explained above, by making CB 1954 to treat solid Walker Carcinoma in Rats, I established the structure activity relationship, and by making AZQ to treat human Glioblastoma, we have demonstrated that all bad genes can be shut off using Aziridine or Carbamate or both as attacking agents to shut off a gene. If you plan to develop drugs to treat other cancers, all we need to do is to identify carriers such as coloring dyes which stains a specific tumor. By attaching Aziridines and Carbamate moiety to carriers to the dyes, we could attack other tumors.

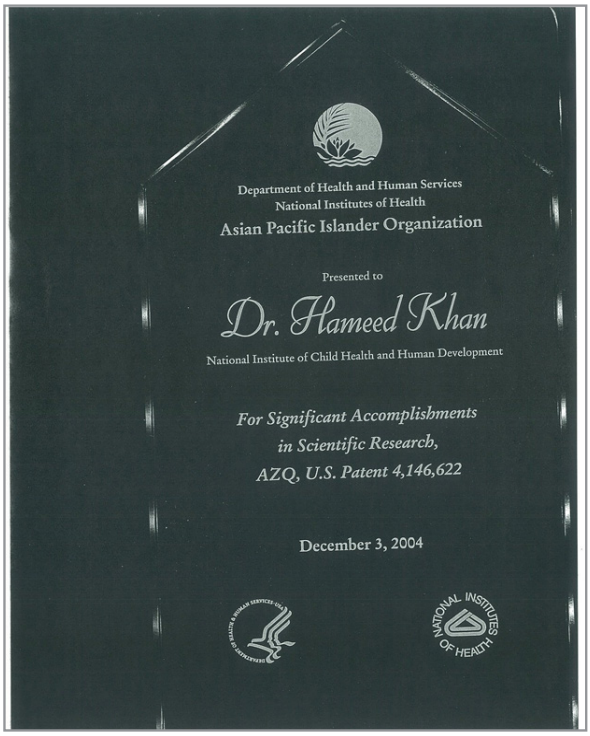
One of the greatest challenges of nanotechnology is to seek out the very first abnormal cell in the presence of billions of normal cells of our brain and shut off the genes before it spread. I worked on this assignment for about a quarter of a century; conducted over 500 experiments which resulted in 200 novel drugs. They were all tested against experimental animal tumors. Forty-five of them were considered valuable enough to be patented by the US Government (US Patent 4, 146, 622 & 4,233,215). One of them is AZQ which not only stops the growth of Glioblastoma,

but also the tumor starts shrinking. For the discovery of AZQ, I was honored with, "The 2004 NIH Scientific Achievement Award." One of America's highest Award in Medicine. I was also honored with the India's National Medal of Honor, "Vidya Ratna" a Gold Medal. (see Exhibits 1,2,3,4)

Exhibit # 1

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

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



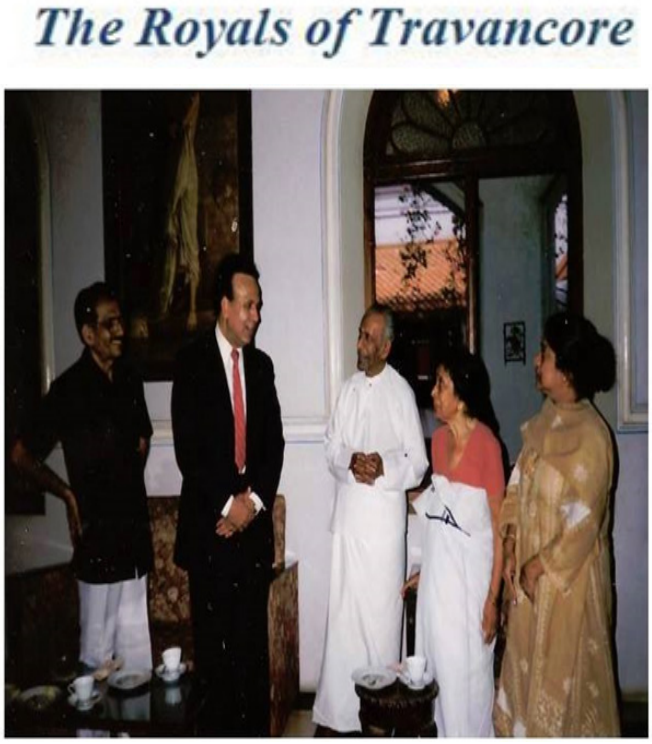
NIH Scientific Achievement Award

Exhibit # 2

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

Discoverer of Anti-Cancer AZQ, After Receiving 2004, Vaidya Ratna,
The Gold Medal, One of India's Highest Awards in Medicine at the Rashtrapathi Bhavan (Presidential Palace), in Delhi, India, during a Reception held on April 2, 2004.

Exhibit # 3



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 was supporting sequencing and mapping of the Genomes, my Institute NICHD was supporting research on Gene Markers associated with diseases.

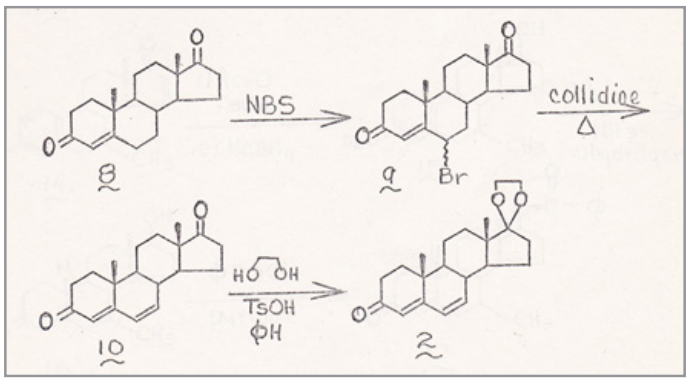
What other Cancers Should we Explore Next?

Could I use the same rationale for treating Breast tumor?

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

Now, I found out that I could go even further by attaching more than four Aziridine and Carbamate moieties to both Male and Female Hormones. Radiolabeled studies showed that male hormone Testosterone has great affinity for female Breast, Ovary, and Fallopian tube cells. On the other hand, Estrogen, the female hormone, has great affinity for male prostate gland. By attaching multiple Aziridine rings and Carbamate ions to both Hormones, I could attack the Breast and the Prostate cancer.

In a Breast tumor, within the start and stop codon, BRCA1 gene has captured over two hundred thousand nucleotide bases. The BRCA1 genes carries about three thousand mutations. These mutations are caused by radiations, chemical or environmental pollutants, viral infection or genetic inheritance. To attack the mutated nucleotides among the three thousand cells in BRCA1 gene, I could use male hormone, Testosterone, and bind multiple radio-labeled Aziridine and Carbamate ions to attack BRCA1 mutations. By using MRI, I could show how many radio-labeled nucleotides were bound to which mutations. Out of seventeen positions available for substitutions on Testosterone [19, 20]. 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]. 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) will use this method to develop drugs to treat all cancers [21-45].

Conclusion

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

The Ideas Expressed in this Article are Mine and Do Not Represent NIH Policy.

References

1. Watson, J. D., & Crick, F. H. C. (1953). A structure for deoxyribose nucleic acid. Nature, 171, 737-738.
2. Hameed Khan. (2021). The Impact of Sequencing Human Genome on Genomic Food & Medicine. International Journal of Genetics and Genomics, 9, 6-19.
3. Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., et al. (2001). Initial sequencing and analysis of the human genome. Nature, 409, 660-921.
4. Collins, F. S., Lander, E. S., Rogers, J., & Waterson, R. H. (2004). Finishing the euchromatic sequence of the human genome. Nature, 431, 931-945.
5. Lindblad-Toh, K., Wade, C. M., Mikkelsen, T. S., Karlsson, E. K., Jaffe, D. B., et al. (2005). Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature, 438, 803-810.
6. Shendure, J., Balasubramanian, S., Church, G. M., Gilbert, W., Rogers, J., et al. (2017). DNA sequencing at 40: past, present and future. Nature, 550, 345-353.
7. Ross, W. C. J. (2015). Chlorambucil. Journal of the American Chemical Society, 136, 5860–5863.
8. Ross, W. C. J. (1953). The Chemistry of Cytotoxic Alkylating Agents. Advances in Cancer Research, 1, 397-449.
9. Ross, W. C. J. (1962). Biological Alkylating Agents. Butterworth, London.
10. Ross, W. C. J. (1949). 43. Aryl-2-halogenoalkylamines. Part I. Journal of Chemical Society, 183.
11. Ross, W. C. J. (1950). 464. The reactions of certain epoxides in aqueous solutions. J. Chem. Soc, 2257.
12. Ross, W. C. J., & Mitchley, B. C. V. (1964). Ann. Rep. Brit. Empire Cancer Campn, 42, 70.
13. Facon, T., Mary, J. Y., Hulin, C., Benboubker, L., Attal, M., et al. (2007). Melphalan and prednisone plus thalidomide versus melphalan and prednisone alone or reduced-intensity autologous stem cell transplantation in elderly patients with multiple myeloma (IFM 99-06): a randomised trial. Lancet, 370, 1209-1218.
14. Cobb, L. M., Connors, T. A., Elson, L. A., Khan, A. H., Mitchley, B. C. V., et al. (1969). 2,4-Dinitro-5-Ethyleneiminobenzamide (CB 1954): A Potent and Selective Inhibitor of the Growth of the Walker Carcinoma 256. BIOCHEMICAL PHARMACOLOGY, 18, 1519-1527.
15. Khan, A. H., & Ross, W. C. J. (1969/70). Tumour-Growth Inhibitory Nitrophenylaziridines and related compounds: Structure-Activity Relationships. PART I. CHEM.-BIOL INTERACTIONS, 1, 27-47.
16. Khan, A. H., & Ross, W. C. J. (1971/72). Tumour-Growth Inhibitory Nitrophenylaziridines and related compounds: Structure-Activity Relationships. PART II. CHEM.-BIOL INTERACTIONS, 4, 11-22.
17. Hameed Khan, A., & Driscoll, J. (1976). Potential Central Nervous System Antitumor Agents: Aziridinylbenzoquinones. PART I. JOURNAL OF MEDICINAL CHEMISTRY, 19, 313-317.
18. Chou, E. D., Hameed Khan, A., & Driscoll, J. (1976). Potential Central Nervous System Antitumor Agents: Aziridinylbenzoquinones. PART II. JOURNAL OF MEDICINAL CHEMISTRY, 19, 1302.
19. Hameed Khan, A. (2021). The Impact of Diagnostic MRI on the Early Detection of Lethal Genes in Human Genome and to Develop Genomic Medicine to Treat Brain Cancers. J Med - Clin Res & Rev, 5, 1-9.
20. Hameed Khan, A. (2021). The Impact of Diagnostic MRI on the Early Detection of Lethal Genes in Human Genome and to Develop Genomic Medicine to Treat Brain Cancers. J Med - Clin Res & Rev, 5, 1-9.
21. Abdul Hameed Khan. (2020). The Impact of Sequencing Human Genome on Drug Design to Treat Oral Cancer. Intech Open. DOI: 10.5772/intechopen.80231.
22. Ala-Eddin Al Moustafa. (2018). Development of Oral Cancer – Risk Factors and Prevention Strategies. Springer.
23. Hameed AK. (2021). The Impact of Diagnostic MRI on the Early Detection of Lethal Genes in Human Genome and to Develop Genomic Medicine to Treat Brain Cancers. J Med Sci Set J Cancer Res, 2023, 16.
24. Leon V Berhardt. (2021). The Impact of Sequencing Human Genome on Genomic Medicine and the Discovery of AZQ (US Patent 4,146,622) Specifically Designed to shut off genes that cause Brain Cancer. Advances in Medicine and Biology, NOVA Medicine and Health, 180, 1-63.

25. Abdul Hameed Khan. (2021). The Rational Drug Design to Treat Cancers: Drug Design - Novel Advances in the Omics Field and Applications. London 117.
26. Hameed Khan. (2021). The Impact of Sequencing Human Genome on Genomic Food & Medicine. International Journal of Genetics and Genomics, 9, 6-19.
27. Hameed Khan. (2021). The Impact of Sequencing Genomes on The Human Longevity Project. J Med - Clin Res & Rev, 5, 1-12.
28. Hameed AK. (2021). The Impact of Diagnostic MRI on the Early Detection of Lethal Genes in Human Genome and to Develop Genomic Medicine to Treat Brain Cancers. J Med - Clin Res & Rev, 5, 1-9.
29. Khan, H. (2021). The Impact of Sequencing Genomes on The Human Longevity Project. J Med - Clin Res & Rev, 5, 1-12.
30. Hameed Khan. (2021). The Impact of Sequencing Human Genome on Genomic Food & Medicine. International Journal of Genetics and Genomics, 9, 6-19.
31. Hameed Khan. (2021). The Impact of Sequencing Human Genome on Genomic Medicine and the Discovery of AZQ (US Patent 4,146,622) Specifically Designed to Shut off Genes That Cause Brain Cancer. ADVANCES IN MEDICINE AND BIOLOGY, 180.
32. Arli Aditya Parikesit. (2021). The Rational Drug Design to Treat Cancers. Drug Design - Novel Advances in the Omics Field and Applications, 95-115.
33. Abbreviated Key Title: EAS J Biotechnol Genet ISSN: 2663-189X (Print) & ISSN: 2663-7286 (Online) Published by East African Scholars Publisher, Kenya.
34. Hameed Khan. (2021). Genomic Medicine: Using Genetic Make-up of the Human Genome, AZQ was Designed to Treat Glioblastoma, the Brain Tumor. Crimson Publishers 6.
35. Hameed Khan. (2021). The Impact of Sequencing Human Genome on Cancer Chemotherapy. J Can Res Rev Rep, 3, 1-10.
36. Hameed Khan. (2021). The Impact of Sequencing Human Genome on Cancer Chemotherapy. J Can Res Rev Rep, 3, 1-10.
37. A Hameed Khan. The Impact of Sequencing Genomes on the Under¬standing of the Origin of Life on Earth. Bi¬omed J Sci & Tech Res, 40.
38. Hameed Khan, A. (2022). The Impact of Sequencing Human Genome on the Genetically Engineered Life. J. Cancer Research and Cellular Therapeutics, 6, 1-16.
39. Hameed Khan, A. (2022). Sequencing the Human Genome to Study the correlation between Genes and Violence, Horizon publishers.
40. Hameed Khan, A. (2022). The Impact of Sequencing Human Genome on the 2nd Genesis on Earth. Journal of Current Trends in Agriculture, Environment and Sustainability, 3, 1-18.
41. Hameed Khan, A. (2022). Assessment of Human Genome Sequencing on the New-World Order. Research Aspects in Biological Science, 4, 84-133.
42. Hameed Khan, A. (2022). The Impact of Sequencing Human Genome on Novel Drug Delivery Method to Treat Cancers. International Journal of Genetics and Genomics, 10, 64-78.
43. Hameed Khan, A. (2022). The Impact of Sequencing Human Genome on New Eugenic & The Impact of Sequencing Human Genome on the 2nd Genesis on Earth. J Cur Tre Agri Envi Sust, 5, 1-21.
44. Hameed Khan, A. (2023). The Impact of Sequencing Human Genome on Idiopathic Diseases. 2. Biomedical Sciences, 5. OAJBS.ID.000554. DOI: 10.38125/OAJBS.000554
45. Hameed Khan, A. (2023). The Impact of Sequencing Human Genome on Darwinian Evolution. J Can Ther Res, 3, 1-26.