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The Impact of Sequencing Human Genome on Cancer Chemotherapy

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ABSTRACT

This abstract describes the rational development of novel drugs design for treating cancers based on the information provided by the sequencing of Human Genome. Out of 24,000 genes in our Genome, sixteen thousand genes code for good proteins that keep us healthy. Six thousand mutated genes are identified which are responsible for causing six thousand different diseases including cancers. The most obvious approach is to design drugs to shut off mutated genes that code for wrong protein. Professor Ross was the first person to use a highly toxic chemical called Nitrogen Mustard developed during the World Wars to shut off a cancer-causing gene. Nitrogen Mustard can cross-link double stranded DNA and shut off a gene. Unfortunately, it has no selectivity. It cross-links all rapidly dividing cells both normal and abnormal cells. This abstract also describes the use of a novel class of drugs called Aziridines which acts as a Prodrug. It does not attack all dividing cells, Aziridines are activated in the presence of acid. As cancer cells grow rapidly, they use Glucose as a source of energy. In cancer cells, Glucose is broken down to produce Lactic Acid. It is the acid that activate Aziridine ring which opens to attack a single strand of DNA shutting off the genes. Over the years, we developed over a hundred analogs of Aziridines and tested against an experimental animal tumor called the Walker Carcinoma 256 in Rats. One compound, Aziridine dinitro-benzamide (CB1854) is found to be seventy times more toxic to cancer cell. Using the same rationale, we translated animal work in human making AZQ (US Patent 4,146,622 & 4,233,215) for treating Glioblastoma, a solid aggressive brain tumor in human. A new approach to treat Breast Cancer is also described.

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Historical Background

We climbed the tallest mountain; we have gone to the bottom of the deepest ocean; we split the heart of atom; we walked on the surface of the Moon and came home safely. Even with the help of the Hubble Space Telescope, we saw the very edge of the Universe at the dawn of time. In short, we have chartered our course from the inner most heart of the atom to the farthest reaches of the Universe. We triumphed in every human endeavor except Life. Now, we have unlocked the secrets of life. On April 14th, 2003, Dr, Francis Collins, the Director of our Institutes, NIH, (The National Institutes of Health) announced that we have read the book in which God Create Life. In one sentences, he described the Human Genome Project (HGP), the greatest biological experiment ever conceived by human mind. It will answer the most fundamental questions we have asked ourselves since the dawn of human civilization. What does it mean to be human; what is the nature of our memory, our conscientiousness; our development from a single cell to a complete human being; the biochemical basis of our senses; the process of our aging; the scientific basis of our similarity and dissimilarity; Similarity that all living creatures from a tiny blade of grass to mighty elephant including man, mouse monkey mosquito and microbe are all made of the same chemical building blocks and yet they are so diverse, no two individual are alike, even identical twins are not exactly identical, they grow up to become two separate individuals.

In 1990, US Congress authorized three billion dollars to decipher the entire Genome within 15 years. US Congress specifically told us that if we don't finish the work by 2005, all funding stop. There were critiques who said that reducing the mystery of life to few organic molecules will not only violate the sanctity of life, but will also violate the laws of God. It would be sin and we would all be in deep trouble after life. We proved them wrong. An army of young intellectuals from 6 industrialized and 20 biomedical centers joined our effort. This effort was led by US and followed by Germany, France, England, China, and Japan and within 13 years the entire book of life was deciphered. It was published in the Scientific Journal Nature and was linked to the Website for all to see. If you a computer keyboard, you have access to all that information. This how we began our work.

A single cell is so small that we cannot even see with our naked eyes. We must use a powerful microscope to enlarge its internal structure. Under an electron microscope, we can enlarge that one cell up to nearly a million times of its original size. Under the electron microscope, a single cell looks as big as our house. There is a good metaphor with our house. For example, our house has a kitchen, the cell has a nucleus. Imagine for a moment, that our kitchen has 23 volumes of cookbooks which contain 24,000 recipes to make different dishes for our breakfast, lunch, and dinner. The nucleus has 23 pairs of chromosomes which contain

24,000 genes which carry instructions to make proteins. Proteins interact to make cells; cells interact to make tissues; and tissues interact to make an organ and several organs interact to make a man, a mouse, or a monkey. In every cell of our body, we carry sixteen thousand good genes, six thousand mutated genes responsible for six thousand diseases and two thousand Pseudo-genes that have lost their functions, during evolutionary time.

Our entire book of life is written in four genetic letters called nucleotides and they are A (adenine), T (thymine), G (guanine) and C (cytosine). These four chemicals are called nucleotide and they 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). In all biological system, from a tiny blade grass to mighty Elephant, including man, mouse, monkey and microbes, all information flows from DNA to RNA to Proteins. 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 RNA (after splicing out the non-coding nucleotides, the RNA is converted to mRNA in which less water soluble methyl group of Thiamine, T, is converted to more water soluble Uracil, U, by replacing Methyl group with a Hydroxyl group) which leaves the nucleus into Cytoplasm where it is translated in Ribosomes into Amino Acids leading to proteins) [1]. Out of the four-letter nucleotide text, every three letters code for an amino acid called Codon. There are 64 codons that code for all 20 amino acids. When thousands to millions of Codons contain information to make a single protein, we call that portion of Codon a gene (Nobel Prize was awarded to Khorana & Nauenberg for making a functional gene).

A Gene is a strip of DNA. The starting Codon for a gene is AUG which codes for the amino acid Methionine after several thousand Codons for different amino acids, comes the stop codon. There are three stop Codons, and they are UGG, UGA, UAG. Once the stop Codon appears, no more amino acids are added, and DNA synthesis stops. If we count all the AT/GC base pairs in a single cell of our body, we will find that there are 3.2 billion pairs of bases present in every cell half inherit from our father and half from our mother. 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.

As I said above, we deciphered all 46 chromosomes. What surprise us most was that our genome contains six billion four hundred million nucleotides contributed by our both parents. 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 the nucleus of 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 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 the following genes on each chromosome:
We found that the chromosome-1 is the largest chromosome carrying 263 million A, T, G and C nucleotides bases and it has only 2,610 genes. The chromosome-2 contains 255 million nucleotides bases and has only 1,748 genes. The chromosome-3 contains 214 million nucleotide bases and carries 1,381 genes. The chromosome-4 contains 203 million nucleotide bases and carries 1,024 genes. The chromosome-5 contains 194 million nucleotide bases and carries 1,190 genes. The chromosome-6 contains 183 million nucleotide bases and carries 1,394 genes. The chromosome-7 contains 171 million nucleotide bases and carries 1,378 genes. The chromosome-8 contains 155 million nucleotide bases and carries 927 genes. The chromosome-9 contains 145 million nucleotide bases and carries 1,076 genes. The chromosome-10 contains 144 million nucleotide bases and carries 983 genes. The chromosome-11 contains 144 million nucleotide bases and carries 1,692 genes. The chromosome-12 contains 143 million nucleotide bases and carries 1,268 genes. The chromosome-13 contains 114 million nucleotide bases and carries 496 genes. The chromosome-14 contains 109 million nucleotide bases and carries 1,173 genes. The chromosome-15 contains 106 million nucleotide bases and carries 906 genes. The chromosome-16 contains 98 million nucleotide bases and carries 1,032 genes. The chromosome-17 contains 92 million nucleotide bases and carries 1,394 genes. The chromosome-18 contains 85 million nucleotide bases and carries 400 genes. The chromosome-19 contains 67 million nucleotide bases and carries 1,592 genes. The chromosome-20 contains 72 million nucleotide bases and carries 710 genes. The chromosome-21 contains 50 million nucleotide bases and carries 337 genes and chromosome-22 contains 56 million nucleotide bases and carries 701 genes. Finally, the sex chromosome of all females called the (X) contains 164 million nucleotide bases and carries 1,141 genes. The male sperm chromosome contains 59 million nucleotide bases and carries 255 genes.

If you add up all genes in the 23 pairs of chromosomes, they come up to 26,808 genes and yet we keep on mentioning 24,000 genes needed to keep us function normally. A gene codes for a protein, not all 24,000 genes code for proteins. It is estimated that less than 19,000 genes code for protein. Because of the alternative splicing, each gene codes for more than one protein. All the genes in our body make less than 50,000 protein which interact in millions of different ways to give a single cell. Millions of cells interact to give a tissue and hundreds of tissues interact to give an organ and several organs interact to make a human. [2-6].

Not all genes act simultaneously to make us function normally. Current studies show that a minimum of 2000 genes are enough to keep human function normally; the remaining genes are backup support system, and they are used when needed. The remaining genes are called the pseudo genes. For example, millions of years ago, humans and dogs shared some of the same ancestral genes; we both carry the same 9,000 olfactory genes needed to search for food in dogs. Since humans don't use all these genes to smell for searching food, many of these genes are broken and lost their functions in humans, but we still carry them. We call them Pseudo genes. Recently, some Japanese scientists have activated the pseudo genes, this work may create ethical problem in future as more and more pseudo genes are activated.

We all carry 220 different tissues in our body and yet we have a single genome, that is the same DNA in every cell. How can all cells carry 24,000 genes and have the same DNA made of AT and GC nucleotides and yet they function in all 220 different tissues? The answer is not all 24,000 genes function in every cell of our body at the same time. Epigenetic answers one the most important questions in the cellular evolution. Small fraction of genes function in different organs and the rest are turned off by either Methylation or Acetylation which serves as Epigenetics agents. For Methylation and Acetylation, the common reagent in the Lab is Dimethyl sulphate or Diazomethane in Sodium Hydroxide for Methylation and Acetic Anhydride in Sulfuric

Acid for Acetylation. The common Epigenetic agents in our body are Folic Acid responsible for Methylation and Acetyl Choline acts as Acetylating Agents. They can Alkylate or Acetylate both DNA or Histone proteins shutting off their genes either temporarily or permanently. Methylation is a common and widely used mechanism for Epigenetic modifications in cells. Abnormal mutations in the Epigenome have been shown to be correlated with many human diseases, including different cancers, autoimmune disorders, neurological disorders (Fragile X syndrome as well as Huntington, Alzheimer, and Parkinson diseases including Schizophrenia).

The Sequencing of the Human Genome which is not only reading the entire book of life of human being letter, by letter, word by word and sentence by sentence, chapter by chapter but also the order in which these letters are arranged called sequencing, is the greatest discovery of all times. The sequencing of the Human Genome will answer the most fundamental questions, we have asked ourselves since the dawn of human civilization; what it means to be human; what the nature of our memory is our conscientiousness; our development from a single cell to a complete human being; the biochemical basis of our senses; the process of our aging; the scientific basis of our similarity and dissimilarity. Similarities that all living creatures from a tiny blade of grass to the mighty Elephants including man, mouse, monkey, and microbes are all made of the same chemical building blocks and yet we are so divers that no two individuals are alike, even identical twins are not identical; they grow up to become two separate individuals.

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

If the fetus carries both bad copies, it will be severely sick. Let me explain with an example how this work will help parents to decide to have a baby even before conception or during pregnancy. A newlywed couple could either conceive a baby either in the bedroom or in the test tubes. If there is a family history of a disease, it is advisable to have in vitro fertilization. The couple gives a sample of eggs and sperms for genetic analysis before conception. Detection kits for several hundred genes are already being developed. The test result may show that the sperm is carrying a genetic defect on Y-chromosome that will make the baby a color blind or give him MS (muscular dystrophy). Doctors will inform the parents whether the child will be incurably blind, or carry a gene for defected heart, kidney, or liver. During the ancient times when Eugenic was at its peak, the authority makes the decision about the fate of the fetus. These days, Parents make the decision to bring this child into this world. How many parents will love to have a blind or permanently sick child in their families? Not many. We must run the census among our people to get the results. It seems reasonable to assume that most parents will not be able to care for that fetus. We may not be able to correct that defect tomorrow, but day after tomorrow may be or in some distant future. We will be able to correct that defect at an enormous cost. Is there any reason for poor parents to keep that fetus alive and grow to full term at an enormous medical expanse? I am sure some rich parents will love to have children at all costs. Such children of rich families will not be burden on society or on our health care system.

Since completing the Human Genome Project, out of six thousand mutated genes, we have already developed over 1500 tests to identify mutated genes, we can provide in vitro fertilization (IVF) of fertilized egg free from all genetic defects. Instead of having children in the bedroom, couples will be able to select out the very healthy eggs and sperms and fertilized them in the test tube and implant them in the mothers. This way we can have the quality control of the babies we bring into this world. The quality control of the population could be accomplished by in vitro fertilization. About 25,000 Mendelian diseases (single gene defects) have been identified and approximately ten thousand are confirmed to specific genes. Developing novel drugs to treat those diseases is expensive and time consuming.

The number and the order of the nucleotide arranged in a Chromosome is called sequencing which determines whether the species is a mouse, monkey, or man. Different species have different number of chromosomes. A Bacterium carries only one chromosome while human being carries 46 chromosomes. Book of life of all living creatures are written in this four-letter nucleotide text. Out of four-letter text, only three letters code for an amino acid, called Codon, and many amino acids interact to make a protein and hundreds of proteins interact to make a cell and billions of cells interact to make a tissue and two hundred and twenty tissues interact to make an organ and several organs interact to make a man or a mouse or a monkey. This four letter nucleotide text provides 64 three letter combination or codons. Mutations or damage to any nucleotide in a Codon, codes for a wrong amino acid which makes a wrong protein which makes us sick. The entire sequence of the Human Genome was published in the scientific journal Nature and it was linked to the website for the world to see. If you have an access to a computer key board, you have access to all that information.

Mutations

The completion of the Human Genome Project opens the path to identifying mutations responsible for causing all six thousand diseases including cancers. Less than two percent of our genome code for protein. The rest is non-coding regions which contains switches, promoters, enhancers etc. Mutations in the coding regions are responsible for causing all cancers. Mutations or damage in the coding region include changes in nucleotide sequence or Chromosomal copy number variations, or double stranded DNA breakage, or methylation. Mutations are caused by several factors including radiations, chemical/environmental pollution, viral infections, genetic inheritance or during replication nucleotide deletion, insertion, inversion, translocations, multiple copying, mismatch nucleotide bases pairing, frame shift mutation, fragile-X, etc. Although they are in the non-coding region, mutations in switches, promotors and enhancer are responsible for causing some unusual diseases.

Cancer Genome Project

The Cancer Genome project is modeled after the Human Genome Project. The Human Genome Project is the book of life of normal cell and the Cancer Genome Project is the book of life of

abnormal cell. The rationale is to examine the order in which the nucleotides are arranged and identify the mutagenic changes in the nucleotide sequence responsible for causing cancers. Once the cost of sequencing comes down to a thousand-fold, as we hope the next generation parallel or nanopore sequencers will do, it would be less expensive to sequence all mutated genes and tumors and then compare them over and over with Reference Sequence and the sequence of the Thousand Genome Project until the exact mutations responsible for causing cancers are localized with precision and accuracy. Once the mutations are localized, we could make a rational approach to design drugs to disrupt the pathways to inhibit the development of cancers. If multiple copies of a nucleotide sequences or N-methylation of nucleotides are observed, it is an indication of the beginning of malignancy. Nucleotide methylation is a natural way of shutting off a gene, we call it Epigenome. Azacytidine is used to remove the methyl group from the nucleotide to activate a gene.

Three years after the completion of the Human Genome Project, in 2006, NIH launched, "The Cancer Genome Atlas" (TCGA), Pilot Project which aims to sequence nucleotide bases of the tumors of most common cancers including Breast Cancer, Ovarian Cancer, Colorectal Cancer, Prostate cancer and brain cancer, Glioblastoma multiforme. The Cancer Genome Atlas (TCGA) project was one of the largest studies ever conducted which included 11,000 patients across 33 tumor types analyzed for key genomic and molecular characteristics. The purpose of the Cancer Genome Project is to Diagnose, Prevent and Treat all these cancers. Sequencing of several tumors showed novel mutations and helped explain pathways and mechanisms in cancer development and their growth and spread. The main conclusion of tumor sequencing is that all cancers are caused by accumulation of nucleotide mutations over the years. There are 220 different tissues in our body and they all could become cancerous if exposed to mutagens. The completion of the Human Genome Project has provided the sequence of all normal tissues. Now, the cost of sequencing has reduced several hundred folds, it is possible to sequence all cancerous tissues, repeated each sequence several hundred times, and compare them with the normal genome Reference Sequence to exactly locate the number of mutations in the gene responsible for a specific cancer. For example, we could easily determine how many mutations in the BRCA1 gene are responsible for causing breast cancer and construct a road-map for disrupting the progress of cancer by designing drugs as personalized genomic medicine that is treatment developed based on the genetic make-up of the disease.

According to Francis Crick's Central Dogma, information in biological system flows from DNA to RNA to Proteins. DNA replicates and is transcribed into RNA, which is translated in the Ribosome into protein, it is the protein which performed all body functions. Normal and abnormal cells both perform the same function, except the abnormal cell performs much faster. Drugs are designed to disrupt the progress of abnormal or Cancer cells by shutting off their genes. Specific drugs are designed to attack the progress of the cancer at all three pathways that is DNA, RNA and Proteins pathways. We have already demonstrated that we have some success by attacking at all three levels.

Protein Pathways were attacked in Breast cancer, by designing a novel drug called Gleevec which is known generically as Imatinib, works by slowing or stopping the growth of certain cancer cells. Gleevec inhibits (or blocks) specific enzymes in the body called tyrosine kinases. Tyrosine kinase enzymes are involved in many cell functions, including cell signaling (communication), growth, and division RNA pathways were attacked by Milo and Craig who designed a double stranded RNA to prevent its translation and shut off RNA function. Double-stranded RNA was substantially more effective at producing interference than was strand individually.

DNA Pathways were attacked by Professor Ross of London University, England. He used a lethal war chemical called Nitrogen Mustard to cross-link double stranded DNA shutting off genes to treat cancers. He introduced a series of highly successful double stranded cross-linking drugs to treat a variety of cancers such as Chlorambucil for treating Childhood Leukemia and Melphalan for treating Pharyngeal Carcinoma.

The biggest killer of the women is Breast Cancer. In 2010, 207,090 women were diagnosed with Breast Cancer and about 40,000 died of the disease and in the same year 1,970 men were diagnosed with Breast Cancer and 390 died of the disease. Although Chromosome-17 (which is made of 92 million nucleotide base pairs and carries 1,394 genes) carries both BRCA1 and BRCA2 genes; mutations on BRCA1 gene is responsible for causing Breast Cancer in both women and men. Mutated BRCA2 gene is responsible for causing Prostate Cancer in Men. The tumor is called Prostate Adenocarcinoma. Human prostate is a walnut size gland located near the male reproductive organ. During the year 2011, the number of cases of men with prostate cancer have increased tremendously; 240,890 new cases were diagnosed with prostate cancer and 33,270 men died of the disease. No new effective drug is available to treat Prostate Cancer either.

In the absence of any effective drugs for treating breast cancer, following preventive measures are recommended: Because of the family history, there are women who are pre-disposed to breast cancer from genetic inheritance. Before the onset of the sign and symptom of the breast cancer such women could be helped by taking preventive measures. If sequencing confirms the presence of mutations, we could offer the options either to undergo hormone therapy or surgery which greatly reduces the risk of breast cancer. This preventive measure keeps these women alive. We have forty thousand deaths due to breast cancer each year. If we want women to live long enough to see their grandchildren grow, they must consider having their Ovaries and Fallopian tubes removed before age 40. After age 40, women do not produce eggs, but the Ovaries are constantly producing Estrogen. High level of Estrogen is constantly stimulating Ovaries and Breast causing cancers.

Viral infection is also responsible for causing cancer. For example, HVP the Human Papilloma Virus is responsible for causing Cervical Cancer in more than 500,000 women and more than 250,000 women die each year worldwide. Other studies are attempting to diagnose mutations responsible for causing Lung Adeno carcinoma, Colorectal cancer, and Ovarian cancer. Once the mutations are localized in these cancers, novel drugs could be designed to attack these tumors.

Genes of Therapeutic Importance

The sequencing of Human Genome showed that each nucleated cell in our body carries 24,000 genes within the nucleus. Sixteen thousand are good genes, six thousand bad or mutated genes and the remaining two thousand are pseudogenes. Good genes produce good proteins which keeps us healthy. Using restriction enzymes, Biotechnology firms have performed genetic engineering that is they cut, paste, sequence, and copy Genes isolated from human genome to produce a variety of proteins at large scale to treat serious diseases. An oversimplified method of large-scale production method is described below; First step is to prepare a restriction site map of a chromosome using EcoR1 type restriction

enzymes which cut chromosomal DNA at various specific site. DNA fragments are purified by Electrophoresis on nitrocellulose gel. A gene is identified by a Start Codon and one of the three Stop Codon. Once purified, genes must be protected from enzymes destruction by making a recombinant DNA by making their Victor. Plasmids, Bacteria or Viruses can serve as Victors. Using a restriction enzyme, we can cut open a circular double stranded Plasmid and insert the desired gene by enzyme DNA ligase. The transgenic Plasmid serves as a Victor. To make large scale, the transgenic Plasmid is harvested in Yeast or Bacterial cells. The pure product is isolated by breaking the transgenic Plasmid with restriction enzymes. Some examples of successfully isolated product of commercial value are described below:

Out of sixteen thousand good genes, that code for good proteins that keep us health, the Biotechnology firms have successfully isolated from our Genome only a few commercially importance genes for large scale production.

Insulin is the most useful protein isolated from pancreas for treating 300 million diabetics around the world.

Human Growth Hormone (GH) which controls our body's growth. GH gene is isolated from the pituitary gland, located at the base of the brain. GH helps children grow taller (also called linear growth), increases muscle mass, and decreases body fat.

Interferons are proteins that are part of our natural defenses which activate our immune system if we are exposed to germs or cancer cells. And they trigger killer immune cells to fight those invaders. Interferons got their name because they "interfere" with viruses and keep them from multiplying. Interferons are glycoprotein cytokine secreted mainly by the kidney in response to cellular hypoxia; they also stimulate red blood cell production in the bone marrow

Plasminogen (PLG) is the major enzyme that degrades fibrin clots and prevent heart attack. In addition to its binding and activating on fibrin clots, PLG also specifically interacts with cell surfaces where it is more efficiently activated by PLG activators, compared with the reaction in solution.

Erythropoietin (EPO) is a hormone that is produced predominantly by specialized cells called interstitial cells in the kidney. Once it is made, it acts on red blood cells to protect them against destruction. At the same time, it stimulates stem cells of the bone marrow to increase the production of red blood cells.

Preventive Actions

To take preventive steps scientists consider it important to conduct a large-scale profiling of tumors to figure out the genetics of cancer. As the cancer cells grow rapidly, the genetic profiles of the cancer cells also change rapidly. For developing novel drugs, we need to know which mutation is more sensitive to attack. After further mutation, the drug will be ineffective to the new mutation; we call this phenomenon the Drug Resistant. In 2008, an International Research Project was launch in which 70 International projects across five continents participated with a shared goal of generating a comprehensive catalog of genomic abnormalities in cancer.

As I said above, to prevent Ovarian Cancer and the Cancer of the Fallopian tube, after the age 40, these organs are not an asset but a burden. After menopause, no eggs are available for fertilization. Women should consider having removed their Ovaries and Fallopian tube. In addition, these organs constantly produce estrogen which also stimulates Breast and ovarian cells and most likely to cause Breast and ovarian Cancer at later age.

The Colorectal cancer is caused by the mutation of two genes namely ERBB2 and IGF2 which are responsible for regulating cell proliferation. These genes are over expressed, and the rate of genetic mutation is abnormally high because normal DNA repair mechanisms are disrupted. Unfortunately, no new drugs are available to shut off these genes. Until we design drugs to shut off these genes, prevention is the best solution. To prevent Colorectal Cancers, all individuals after age 60 and above should have Colonoscopy done to remove all polyps. Colorectal cancer develops slowly. As polyps age, they start leaking blood at microscopic level. Most individuals do not notice blood in their feces: they grow weaker and begin to age pre-maturely. Before the polyps become cancerous and start bleeding, they should be removed and should be checked every three years for any additional polyps.

Therapeutic of Cancer

Whole genome sequencing and exome sequencing, determine copy number changes. For example, when we compare the normal colon genome with the colon tumor genome, it was observed that when the copy number increases, the tumor increases, copy number changes will show the progress of the cancer. Comparison with the 500 genomes with the patients confirms this observation. High number copy changes have high mutations and have number of tumors. In addition, comparing Colon cancer, we found the normal cells have one mutation per one KB (kilo bases) while malignant cells have as many as 800 mutations per thousand A-T & G-C base pairs. Other sources mutations include, copy number changes, DNA mismatch repair system, Intra-chromosomal rearrangement, double-stranded break and Inter-chromosomal rearrangement or recombination. As the disease progress enormous number of rearrangements in a small segment of DNA is observed.

To develop new drug for treatment, you need to know all this information. Will the animal respond or develop resistance or to turn on or turn off the pathways or causes structural aberration? If the cancer genome does not match to human genome, we need to know the changes responsible for causing this change. To conduct analysis, we need to know the role of genetic and genomic changes responsible for causing cancers. Unless we understand the disruption of different chemical pathways and how these changes are responsible for causing diseases, we cannot design drugs to stop the progress of cancers.

Out of 24,000 genes in our Genome, six thousand mutated genes are responsible for causing diseases and they could be identified. Once identified, they could either be replaced (by Gene Therapy) or shut off (by Drug Therapy).

Gene Therapy

One of the most successful examples of replacing a bad gene with a good gene is Gene therapy which requires replacing a mutated gene with good gene from the same patient. Good gene is recombined with plasmid and is cloned in stem cells. Harvest the hybrid clones. Using the restriction enzyme to cut, paste and copy the good protein and give this protein back to the same patient. French Anderson and Mike Blaise while working in our Labs at NIH isolated WBC (White Blood Cells) from a girl named Ashanti DeSilva, who was suffering from the Severe Combined Amino Deficiency Syndrome (SCAD), an illness which destroys the immune system. Such patients are required filtered air to survive. This disease is commonly known as the Bubble Boy

Syndrome. Mutation in the Adenosine deaminase genes does not clear excessive amount of adenosine from the blood resulting in the loss of patient's ability to fight off infectious diseases. Using the restrictions enzymes, French Anderson & Mike Blaise cut, paste and copy Adenosine Deaminase Gene in Adeno viruses and infected WBC obtained from Ashanti DeSilva, harvested outside, and injected back in Ashanti. These transgenic WBC become functional producing enzyme Adenosine deaminase clearing excessive amount of Adenosine. Ashanti's immune system becomes functional and she lives a normal life. There are more than five thousand genetically modified children who are living a normal life. Several clinical trials of gene therapy are underway. Gene therapy is successful for treating a single gene mutation disease, unfortunately, diseases with multiple genetic defects such as Cancers, Diabetes and Cardio-vascular diseases would not respond to Gene Therapy. For multiple genetically defected diseases, Gene Therapy will not work; Drug Therapy will work.

Designing Drugs to Shut Off a Specific Gene

Although nucleus of every cell in our body carries a completer Genome carrying all 24,000 genes, we found that not all genes function simultaneously in every cell. During cell differentiation, different cells take different roles. Only specific number of genes are turned on to make Liver, Lungs or Kidneys and all other genes are turned off. Nature's way of stopping gene function is by methylation called Epigenetic. In vivo methylation is carried out by Folic Acid to stop gene function. By using Azacytidine, we can remove the methyl group and restore gene function. Protein function can also be stopped by Acetylation. Nature uses Acetyl Choline to stop the synthesis of Histone protein. In the Lab, we could use Acetic anhydride in Sulfuric Acid to acetylate the Histone Protein.

The greatest challenge in the Lab is how could we design drug to shut off a specific gene which is responsible for causing cancer and not attack other normal genes. This section describes the synthesis of highly toxic drug Nitrogen Mustard which shut off genes of all dividing cells. It also describes the synthesis of non-toxic Prodrugs like Aziridines and Carbamates which are activated in acidic media to attack only the cancer cells.

Drug Therapy

Drug therapy is used to treat diseases with multiple Genetic defects. Professor Walter Charles James Ross of the University of the London, England, was the first scientist to use Nitrogen Mustard, a war chemical, to treat Cancer. He observed that soldiers who were exposed to Nitrogen Mustard, during WWII were dying of freezing cold. Blood analysis of those soldiers showed a sharp drop of White Blood Cells (WBC) from 5000/CC to 500/CC in the mustard poisoned solders. Children who were suffering from Childhood Leukemia showed a sharp increase in WBC. The pre-mature lymphocyte counts was up from 5000/CC to 90,000/CC. Ross wondered if lymphocyte count could be brought down to normal level in these sick children by using controlled amount of Nitrogen Mustard. It was indeed found to be true. It was the first successful example of Cancer Chemotherapy of using poisonous chemicals to treat cancers. Ross and his group made hundreds of the analogs of Nitrogen Mustard. Several of those analogs of Nitrogen Mustard are used for treating various types of Cancer. Among his most successful analogs of Nitrogen Mustard are used today are Chlorambucil for treating Childhood Leukemia and, Melphalan for treating Pharyngeal Carcinoma etc [7-13]. C-14 radio labeled studies showed that the two Chlorine atoms from the Bis-di chloroethyl amino group of the Nitrogen Mustard are eliminated by enzymes which generate two positively charged Carbonium ions which attack the electron rich N-7 Guanine on both strands of the double stranded DNA cross-linking the two strands shutting off the gene.

During cell replication, each strand of the double stranded DNA must separate, and each serve as a template for the formation of the two daughter cells. After cross-linking, the two strands of DNA do not separate; the cross-linked cells do not grow and the cell die. Since all growing cells, normal as well as abnormal, cell divide, all dividing cells are covalently bond by the Nitrogen Mustard molecule. Since cancer cells grow faster than the normal cells, a ratio of the toxicity from cancer cell to normal cell is measured and its ratio is called the Therapeutic Index (T/I). The higher the TI is considered, the more toxic to the cancer cells. Toxicity measurement against a solid tumor called the Walker Carcinoma 256 in Rat which showed that Nitrogen Mustard has a (T/I=10) that it is ten times more toxic to cancer cells when compared to normal cells. All cross-linking analog of Nitrogen Mustard is found to have a T/I=10.

Professor Ross' group is made of some of the finest scientists. Over the years, they made hundreds of analogs of Nitrogen Mustard in an attempt to increase their toxicity. The more toxic a substance is the less amount could be used to treat cancers. I was honored to be Professor Ross' graduate student, his Post-doctoral Fellow and his special assistant. I was in his Lab for almost ten years.

One of the contemporary of Professor Ross was Dr. James Warwick who was also a faculty member of the Post-graduate Center of the London University, The Chester Beatty Cancer Research Institute at the Royal Cancer Hospital. Both Ross and Warwick were working in the same building on different floors. During a radio labeled study of Nitrogen Mustard, Dr. Warwick discovered that Nitrogen Mustard do not attack both strands of DNA simultaneously. In fact, one arm of the Nitrogen Mustard, binds to a single strand of DNA while the second arm binds to the Nitrogen atom of the same Nitrogen Mustard molecule forming a three-member intermediate ring called Aziridine. Warwick was unable to isolate Aziridine intermediate because it is sensitive to acid and growing cancer cells uses Glucose as a source of energy which is decomposed to produce Lactic acid which destroys Aziridine moiety. Since Warwick died in unusual circumstances (scientist bury their dead quietly), no one in the Royal Cancer Hospital was working on the Aziridines during the past 40 years.

Rationale for Developing Aziridine Analogs as Anti-Cancer Prodrugs

Since all members of Ross's group were making analogs of Nitrogen Mustard as cross-linking agents to bind both strands of DNA, I asked Professor Ross if I could make the analogs of Aziridine as a single strand DNA binding agent. We both agreed that single stranded DNA binding agents would be half as toxic as the double stranded DNA binding agents like Nitrogen Mustard. Could we increase the anti-tumor activity by using single stranded Aziridine analogs? Making Aziridine analogs for my graduate work was a risky business. Although I accepted the assignment, my doctoral thesis has an uncertain future. Little did I know that the greatest surprise was waiting in my future which resulted in the synthesis of an analog of Aziridine di Nitro benzamide called CB1954, which showed a TI of 70 [14]. It was seventy times more toxic, highest ever recorded to cancer cell when compared with any analogs of Nitrogen Mustard against the experimental solid tumor, Walker Carcinoma 256 in Rats.

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

As a part of my Doctoral Thesis, I attached alkylating Aziridine to dyes like Dinitro Benzamide to attack the DNA of an experimental animal tumor called Walker Carcinoma 256. Analogs of dinitro benzamides are Prodrug, they have to be activated in the acidic media. Cancer cells grow faster than normal cells, they use more Glucose as a source of energy. Glucose breaks down to produce Lactic Acid. The Aziridine moiety is unstable in acidic solution. The Aziridine breaks down to open its ring to produce a positive Carbonium Ion. The Carbonium ion is extremely reactive; it binds to a single strand of DNA shutting off its gene. It preferentially binds to N-7 of Guanine killing the tumor cells. Professor Ross and I have demonstrated the attack on N-7 of Guanine using the radio labeled studies. As a part of my Doctoral and Postdoctoral studies, over the years, I made 120 Dinitro-Benzamide derivatives for testing against Walker Carcinoma 256 in Rats [15,16].

To test against a variety of tumors, from our Labs at the Royal Cancer Hospital, University of London, England, I had sent to America at the NIH (National Institutes of Health) over 120 drugs for NCI (National Cancer Institute) screening program. NCI honored me with the Fogarty International Award to come to America to continue my work with Aziridines translating the animal work to Humans. As I said above, NIH is the largest biomedical center in the world. It has unlimited facilities (chemicals, equipment, and personnel). Twenty-one thousand best and brightest scientists selected from Ivy League schools work in 26 Institutes in more than three thousand labs. I was honored to join this group at NCI. NIH has been my home for over a quarter of a century.

Brain Cancer Chemotherapy

I used the same rationale to continue my work in America. I brought the idea from London University of attacking one strand of DNA using Aziridine, but I do not want to use the same dye Dinitro benzamide or its analogs. One day, I came across a paper which described that methylated C-14 radiolabeled Quinone crossed the Blood Brain Barrier. When injected in mice within 24 hours, the X-ray showed that the entire radioactivity was concentrated in the Brain. I knew that Glioblastoma multiforme, the human brain tumor, is a solid aggressive tumor like Walker Carcinoma in Rats. I decided to use Quinone moiety as a carrier for Aziridine rings to attack Glioblastoma. I was delighted when I realized by introducing just one Aziridine and one Carbamate moiety to Dinitro Benzine ring, I produced so toxic compound against tumors that its toxicity could not be measured. For safety reason, this work was discontinued at the London University, but was allowed to continue in America at NCI (National Cancer Institute). I made a series of Quinone analogs of Aziridine and Carbamate to attack the brain tumor.

As I said, Glioblastoma multiforme is a solid brain tumor; it is an aggressive tumor. It grows so rapidly within months; it grows so large. Its sheer size will crush the wiring diagram; crush the neuronal circuits and crush synapses and most patients internally bleed to death within 14 months. One of the greatest challenges of the nanotechnology is to seek out that very first abnormal neuron within billion normal neurons and shutting off that gene that causes Glioblastoma multiforme. When it was confirmed that the Radio labeled Quinone cross the Blood Brain Barrier, by attaching the Aziridine and Carbamate moieties to Quinone molecule, I could design Di-Aziridine Quinone Di-Carbamate (I named it AZQ) which serves as a Prodrug. It remains inactive in neutral and basic media but are highly sensitive to acid; they become activated only in the presence of acidic media. Once the malignant cells of the Glioblastoma multiforme begin to replicate and grow, they use Glucose as a source of energy. Glucose decomposes to produce Lactic acid. The acid molecule will activate Aziridine ring and the Carbamate moieties to generate Carbonium ions which will attack a single strand of DNA shutting off the gene [17-19].

Over the years, I conducted over 500 experiments which resulted in 200 novel drugs which were tested against experimental animal tumors. Forty-five of them were considered valuable enough to be patented by US Government (US Patent 4,146,622 & 4,233,215)). One of them called AZQ acts as a silver bullet. Glioblastoma was not only stop growing, but also start shrinking. For the discovery of AZQ, I was honored with the "2004 NIH Scientific Achievement Award" one of America's highest award in medicine and I was also honored with the "Vaidya Ratna" a Gold Medal, one of India's National Medal of Honors. (Exhibit: 1, 2, 3 & 4).

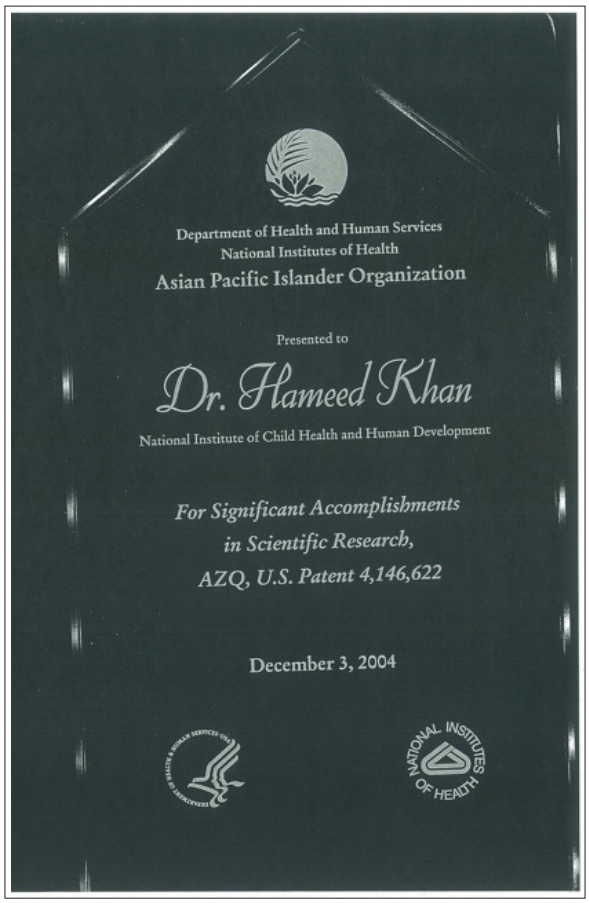
Exhibit: 1

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), 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.

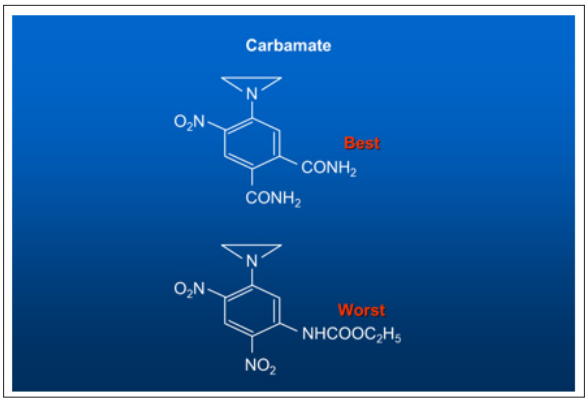
Citation: A Hameed Khan (2021) The Impact of Sequencing Human Genome on Cancer Chemotherapy. Journal of Cancer Research Reviews & Reports. SRC/JCRR-153. DOI: doi.org/10.47363/JCRR/2021(3)146

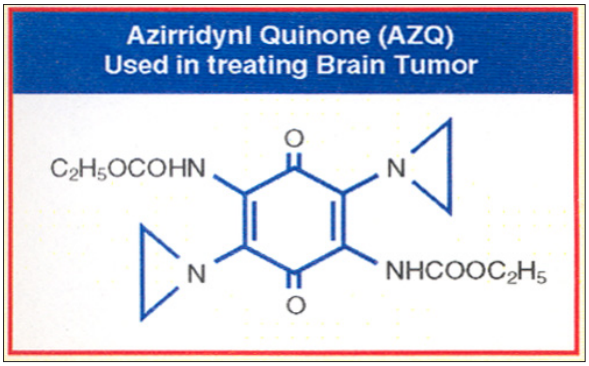


His Excellency, Dr. A.P.J. Abdul Kalam,
The President of India
Greeting
Dr. A. Hameed Khan

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

Exhibit: 3
Single Strand DNA Binding Aziridine and Carbamate





U.S. Patent 4,146,622

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.

Designing Future Drugs

According to the World Health Organization, approximately 1.3 million new cases are diagnosed with breast cancer of which 450,000 died worldwide annually. Breast cancer is the most common cancer among women. Most cases are sporadic, meaning there is not a family history of breast cancer, as opposed to genetic, where genes predispose a person to the disease. Besides genetic inheritance, the sporadic cases of breast cancer are caused by the

exposure to radiations, chemical/environmental pollution, viral infection, genetic inheritance etc. Men can also develop breast cancer, but it accounts for less than 1 percent of breast cancer cases.

Mutation in the BRCA1 gene is responsible for causing breast cancer in both men and women. The abnormal growth begins in the tissue of the breast and then spread rapidly. It is the second major cause of death of women in America. Mutated BRCA2 gene is responsible for causing prostate cancer in men and both BRCA1 and BRCAII genes are identified on Chromosome-17. There are 92 million nucleotide base pairs on Chromosome-17, which carries 1,394 genes. Of all the cancers, Breast Cancer is the biggest killer of women. During 2021, in the United States, about 255,000 cases of breast cancer are diagnosed in women and about 2,300 in men. About 42,000 women and 500 men in the U.S. die each year from breast cancer. The current treatments of Breast Cancer is Surgery, Radiation Therapy and Chemotherapy.

In performing surgery or Mastectomy, once the breast cancerous tumor is operated and removed, two problems remain unsolved. First, the normal and abnormal cells are integrated. There is no line of demarcation between normal and abnormal cells. One cannot remove every embedded malignant cell from the normal breast cell. After Mastectomy, the remaining primary malignant cells will become Metastatic and will return within three years as metastatic cancer and will kill the patient. Second, soon after Mastectomy, we must design drugs not only to treat the malignant cells imbedded in the breast, but also to attack the returning malignant cells.

In the last section, I am proposing how to attack not only the existing primary embedded mutated cells, but also to attack the metastatic breast cancer cells returning within 3-5 years. During a series of experiment, it was discovered that radio-labeled male hormone, Testosterone, when injected into female mice, was attracted to female organs. Within 24 hours of injecting radiolabeled Testosterone, X-ray detected radioactivity at breast, ovary, and fallopian tube of female mice. On the other hand, when radio-labeled female hormone Estrogen was injected to male mice, within 24 hours, X-ray showed radioactivity on the prostate glands of male mice. We immediately realized that hormones could act as carriers for Aziridine and Carbamate moiety to attack the tumors of the sex organs.

By using the same rationale as was used in the synthesis of AZQ for treating Glioblastoma multiforme, we could attach Aziridine and Carbamate moieties to both male and female hormone molecules. We could attack both male and female reproductive organs tumors provided they grow and use Glucose as a source of energy and produce Lactic acid as their by-product. It is acid which trigger Aziridine and Carbamate to produce a Carbonium ion which attacks tumor DNA shutting off the gene. As opposed to cross-linking DNA, Aziridine and Carbamate Carbonium ion would attack a single strand of DNA shutting off both BRCA1 and BRCA2 genes.

In the past, we have made several attempts to understand the cancer, until we completed the HGP (The Human Genome Project) and discovered that damage in the genetic code caused the mutation which causes the abnormal growth. Cancer Genome Project confirms that cancer is a genetic disease and mutations in the genetic code are responsible for causing cancers. Besides brain and breast, mutations in germ line cell are detected in organs such as Colon or in somatic cells which causes cancers. Mutations in tumor suppressor genes and oncogenes are also found in cancer cells. Besides damage to DNA, other changes responsible for causing cancers include copy number changes, DNA-methylation changes and the changes in the gene expression.

The completion of the Human Genome Project provided the blue print of the normal healthy cell and the Cancer Genome Project provided the blue print of the abnormal cell. Comparison of these two Genomes will provide the sites of mutations responsible for the abnormal growth. It also helped develop a series of technologies alongside to help sequence the genome faster and cheaper. The goal of the future study is to sequence as many solid tumors as possible and compare them with the 1000-Genome Project to identify mutations with precision and accuracy and then design drugs to shut off those genes.

Breast & Prostate Cancer Chemotherapy

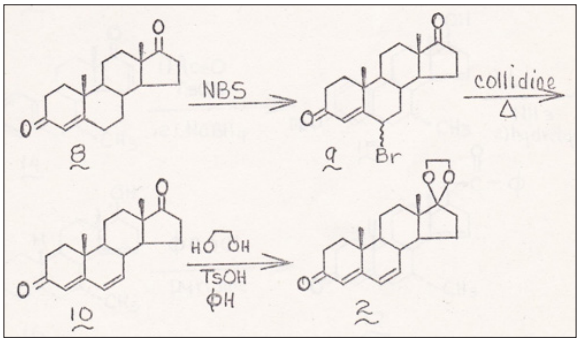
Although BRCA1 gene located on Chromosome-17 (which is made of 92 million nucleotide bases carrying 1,394 genes) has been identified years ago, we wonder why it has been so difficult to treat Breast Cancer. By the time the Breast Cancer diagnosis is confirmed in a patient, the BRCA1 has accumulated more 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 for 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 two Aziridine and two Carbamate moieties to both Male and Female Hormones. Once radiolabeled studies confirmed that male hormone Testosterone has great affinity for female Breast, Ovary, and Fallopian tube cells. On the other hand, Estrogen, the female hormone, has great affinity for male prostate gland. By attaching multiple Aziridine rings and Carbamate ions to both Hormones, I could attack the Breast and the Prostate cancer.

In a Breast tumor, within the Start and Stop codon, BRCA1 gene has captured over two hundred thousand nucleotide bases. The BRCA1 genes carries about three thousand mutations. These mutations are caused by radiations, chemical or environmental pollutants, viral infection, or genetic inheritance. To attack the mutated nucleotides among the three thousand cells in BRCA1 gene, I could use male hormone, Testosterone, and bind multiple radio labeled Aziridine and Carbamate ions to attack BRCA1 mutations. By using MRI, I could show how many radio-labeled nucleotides were bound to which mutations. Out of seventeen positions available for substitutions on Testosterone. There are only three positions that is 1, 3 and 17 positions are available for substitution on Testosterone ring system. The great Carl Djerassi showed how to activate position 9 and 10 by reacting with Bromo-acetamide (see structures below) which introduce a Bromo ion on position 10 which could be de-brominated by Collidine to introduce a 9,10 double bond which I could further brominate to produce 9,10 dibromo compound [20]. 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. These compounds are non-toxic Prodrug. When activated in acidic medium, they could create havoc of tumors.

We could use similar rationale to treat Prostate Cancer. Prostate is walnut size gland in the male reproductive system. The Prostate Cancer is called the Prostate adenocarcinoma. As I said above, the mutation in the BRCA2 gene is responsible for causing Prostate cancer. After diagnosis, prostate cancer spread fast, and most patients die within two to three years. Every year almost a quarter million men would be diagnosed with prostate cancer and more than thirty-three thousand would die from Prostate Cancer. High level of male hormone testosterone is responsible for causing mutation in SPOP and FOX1 genes.

Mutations responsible for causing prostate cancers to have been identified in 333 patients and 90 percent of the prostate cancers are identified as clinically localized tumors. Sequencing prostate tumor showed that these alterations include DNA methylation which inhibits gene expression, somatic copy-number alteration, and the number of a gene in a cell. This diagnosis will lead to their treatment.



Conclusion

Over a quarter of a century of our experience, we demonstrated that using the dye dinitro benzamide and by attaching Aziridine moiety, we made CB1954 that could wipe out an aggressive, solid tumor like Walker Carcinoma 256 in Rats and by using Quinone we made AZQ as a carrier for Aziridine, we could transport alkylating Aziridine and Carbamate across Blood Brain Barrier and attack Glioblastoma in humans which not only stop growing Glioblastoma, but also start shrinking the tumor. We need to train an army of young scientists to think rationally to design drug to treat cancers based on the Genome Sequencing and identifying mutations on all abnormal cells. I have been invited to speak on rational drug design at the International Conferences in eleven different countries. My aim is to train a new generation of scientists to identify harmful mutations. Once identified, we can either use highly toxic pure drug like Nitrogen Mustard to shut off the gene or we could use nontoxic Prodrug like AZQ to treat cancer.

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