**Patrick Arbuthnot**
**Director: Antiviral Gene Therapy Research Unit**
**School of Pathology**
**Health Sciences Faculty**
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Thank you for the kind introductions, Professor Laburn and Professor Wadee. Good evening ladies and gentlemen and welcome to this lecture. The letter that I received from Dr Goolam of the office of professor Ballim, which provided the guidelines for this lecture, made two points that I thought were particularly important. Firstly, there is no restriction on the content of the talk. As mentioned by Professor Laburn, it is customary for an incumbent to give an inaugural lecture about her or his field of work and that is indeed what I plan to do. This lecture will be a personal account of research that I do in gene therapy, and the personal aspect will deal with my interpretation of the importance of the field, the area that I work in, the problems that face advances in gene therapy and the reasons for being interested in the topic. The second guideline that I thought was important was that the lecture is a public one, and should therefore be accessible to lay people. Unfortunately scientists are generally not very good at communicating their work to the general public, which is unfortunate. In my experience lay people are very interested in hearing about science and the fault lies with scientists who communicate their work poorly to a wide audience. The quote of the renowned science fiction writer, Isaac Asimov, is particularly relevant to this and is projected on this slide. He stated that

'The saddest aspect of life today is that science gathers knowledge faster than society gathers wisdom'.

Science of course has a lot to offer the public and effectively communicating the usefulness of science is very important. Scientists researching topics in the health-related fields have valuable practical benefits as a result of creation of interventions, methods, or strategies to treat disease. Moreover, the scientific method has a social function that is useful to nurture an ethic that encourages critical and creative thinking. This presentation is therefore primarily aimed at conveying insights about gene therapy to people who do not

have a background in biology or medicine. The topic of gene therapy is rather technical in nature and I am therefore obliged to simplify some of the complex concepts. Also, parts of the talk will necessarily be of a didactic nature, which is important to provide the framework for discussing general issues of the significance of gene therapy in South Africa.

The content of this presentation will be divided into 5 main parts, which are

1. Why genes are important in normal and disease biology
2. What is gene therapy?
3. Using silencing techniques to counter hepatitis B virus infection
4. The Challenges and
5. Relevance of gene therapy research

The critically important role of genes in life as we know it is related primarily to the information-carrying capability of genes. A gene is essentially a unit of information within an organism, whether that organism is a human, virus or bacterium. You may ask why this information carrying capability is all important, and the reason is that this information may be transformed into function. The unraveling of the way in which a gene is transformed into a function is the major discovery of biology of the 20$^{th}$ century. The essence of this process is summarized diagrammatically in this slide. Molecular biologists have borrowed terms form language to describe the process. Genes, which comprise DNA, are transcribed to form RNA, which in turn is translated into proteins. The general concept is that DNA is an excellent information carrying molecule, while proteins are poor information carriers, but have enormous structural diversity, which is good for their role as functional molecules. Proteins are capable of folding into an enormous variety of structures, which is critically important for their role as functional units of living organisms. The collection of functions acting in concert is what makes humans what they are, and it is critically important that functions are carried out in a regulated and precise manner. Disruptions to the ways in which genes

function may result in disease, and this is what gene therapy generally aims to correct. The summary of the role of genes in the functioning of organisms is summarized well by Sydney Brenner, a graduate of this university, who was at the forefront of research during the 1950s and 1960s which was the golden age of biology research. He said **'The total explanation of all organisms resides within them (genes)…. Ultimately, the organism must be explicable in terms of its genes….'**

To place the role of genes into a context that is readily tangible, we should consider superficially the way in which humans function, and take as an example two fairly typical South Africans.

The two individuals shown on the slide may be known to you as the lead artists of the group called 'Die Antwoord'. The style of music is very alternative and quite difficult to categorise, but may be called a type of Afrikaans rap music. The lady on the left with the short fringe, is called Yolandie Vi$$er. Importantly her surname is spelled with dollar signs and not 's's'. The man on the right with the interesting inkwork is called 'Ninja'. I am informed by my brother who is in the audience and knows about these things, that Ninja does not have this name because he is a martial arts expert. Rather it is an acronym for his state at the time before setting out on his career in music. Ninja apparently stands for No Income, No Job (or) Assets. Despite this bereft material state, we can be sure that Ninja does have the organs that make him a human like all of us in this room. He has a brain to enable him to think and compose his music, muscles to allow him to move his limbs and dance, lungs for breathing or controlling his singing, a heart and large blood vessels to pump blood around his body to provide oxygen and energy to all of his tissues, a liver to control his metabolism and carry out some detoxifying functions (which may be important to him), as well as intestines to digest the food that he takes in. Collectively these organs all function in specific ways to carry out essential functions that are required for humans to exist. These organs have the same genetic material, but there is a programme of gene activity to make organs function in a particular way.

If we consider the liver, and this is generally true for all organs in the body, there are collections of cells that make up the organ. These are microscopic units that vary in different organs and are collectively responsible for the functions of an organ. The most abundant liver

cells, which are called hepatocytes, are clear to see in this slide. They are stained pinkish and have a bluish nucleus. The cells are also arranged in a specific architecture, which is important for their function. Genetic information, which is arranged in chromosomes, is located in the nucleus. We humans have 46 chromosomes, and 23 come from each of our parents. Chromosomes are structures comprising highly condensed arrangements of DNA. The DNA is a linear molecule that needs to be folded in a highly organized manner to fit within the nucleus of a cell. This is a remarkable feat if we are to consider that the human DNA of all of the chromosomes in one cell, if it were to be extended and aligned end to end, would be about 2 m long. DNA of course is the basic information-containing material of genes and the length of the DNA correlates with the amount of information that is contained in the DNA. Discovery of the double helical structure of DNA by James Watson and Francis Crick, and the insights that were derived from this discovery, was the major achievements of biology in the 20$^{th}$ century. Understanding how genes operate was the major driving force behind advances in the second half o the 20$^{th}$ century and continues to underlie research in field such as gene therapy. A point that is often misunderstood is that Watson and Crick did not discover DNA, it was the structure that they discovered. DNA had in fact been discovered in the 1869 by Friedrich Miescher. He was a Swiss scientist and was working on the chemistry of white blood cells. He chose to study these cells as they are abundant in pus and he had convenient access to bandages from a hospital near his university. He was able to isolate the DNA material, which he called ‘nuclein’, from salmon and other fish sperm too. Another important discovery in the history of DNA was the demonstration by Oswald Avery and his colleagues (working at the Rockefeller Institute in New York) that DNA was responsible for inheritable characteristics. The major contribution of unravelling of the structure by Watson and Crick was that it provided the molecular insights that are required to understand how DNA works as an information-carrying molecule. The iconic publication of their work was in a one page article that appeared in Nature on the 24$^{th}$ of April in 1953. This article is interesting for how simple it is according to today’s standards. There is one hand drawn figure with a very brief legend, and the content of the text is very brief.

There are a few points made in the second half of the article which hinted at the biological significant of their discovery. The first was the statement that

**‘The sequence of bases in a single chain does not appear to be restricted in any way’.**

The implication of this is that the information is the sequence of bases and is unrestricted. The second rather understated point, which was made in the concluding paragraph, was that

**‘It has not escaped our notice that the specific base pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.’**

There was a follow up article that appeared on the 30th of May 1953, and this publication fleshed out Watson and Crick’s interpretation of the biological significance of the structure they had elucidated. The information on DNA structure led logically to understanding the mechanism by which information is transformed to function, in other words transcription and translation to make proteins. Of course, the understanding also meant that insights were gained into the ways on which disruption to genes may cause disease, and this was the origin to gene therapy as a field of study. Naturally, when the structure of DNA was deciphered and an understanding was gained into how changes to gene function may cause disease, a focus of attention was the development of methods to correct or counter these disruptions for therapeutic effect, and this was the origin of Gene Therapy.

We now know that there are approximately 3 billion base pairs in the genetic makeup of humans. The base pairs comprise pairings between A and T or G and C and it is the sequence of these nitrogenous bases which confer information on the DNA molecule. We do in fact have 2 copies, one from our mothers and one from our fathers, so each cell in fact has 6 billion base pairs. You will notice that I included a scale on the diagram, which indicates that there are 3.4 nm (3.4 billionths of a metre) for every 360 degree turns of the helix which also comprises 10 base pairs. You can do the sums to work out why there are 2 m of DNA in a single nucleus. Human DNA encodes about 23 thousand genes, and it is the integrated expression of all of these genes, to encode functions, that accounts for the way in which cells within organs function. So, to take the path in reverse, the panel of genes which is switched on in a group of cells confers a specific set of functions within that cell (Sydney Brenner’s genetic grammar), which in turn is important for the working of the organ in which the cell is

located and then the function of the entire body. A recent very exciting and promising field of research has been the topic of reversing the characteristic of differentiated cells to form stem cells and then reprogramming these cells to carry out other functions.

If we consider the details of what a gene is and how it functions, it is essentially a long piece of DNA, which encodes different types of information. Firstly, there is a section of the DNA which defines the start of the gene, and there is another part that defines where the stop occurs. These signals direct the protein enzyme, which is called RNA polymerase, to transcribe the genetic information to form a messenger of the gene, or messenger RNA. After this step, the messenger RNA is translated into a protein, which as you now know is the functional component of a gene. Several different types of disruption to gene function can occur and these may be in the section that is responsible for the starting or stopping of gene expression to diminish production of a protein and also the region that encodes the protein functional unit. Commonly cancers result from changes in the protein coding regions of genes. For example, cigarette smoking can cause chemical changes to DNA, which alter the function of certain important genes. Also introduction of exogenous genetic material, such as occurs during a viral infection, may result in disruption to gene expression in a cell. Gene therapy as a field of work aims to develop methods of reversing these disruptions. When one considers that all biological processes are dependent on genes to function, it is evident that if one were to be able to restore genes' functions when disrupted, or counter the effects of extraneous genes such as those from viruses, then one would have a very powerful method of treatment.

Despite these fairly obvious and significant objectives, the definition of gene therapy is perhaps not as clear as on may initially think. It cannot really be defined as any procedure that entails the alteration of gene expression. These are many so called small molecules such as hormones, which change gene expression, but these drugs are not considered to be gene therapy agents. A good example is use of anabolic steroid hormones to build muscle mass. These hormones switch genes on that are responsible for making the proteins that give muscles their strength, but this is not gene therapy. This example is quite a dramatic one, but it is important to appreciate that by far the majority of genes are not amenable to therapeutic manipulation by small molecule drugs. A major critically important advantage of gene therapy drugs, which is theoretical at the moment but will no doubt be realised, is that gene

therapy enables rational design of drugs. The rational design is based on the use of selected nucleic acids to have their therapeutic effect. Originally, gene therapy was considered to entail the introduction of DNA into a cell, but more recently, RNA or modified DNA or RNA may also be gene therapy drugs. So the definition that I favour is that gene therapy is '**Gene Therapy is a procedure that employs nucleic acids to achieve a therapeutic effect'.**

The main types of disease that gene therapy has been applied to has been the correction of

1 gene defects that cause inherited diseases, and an example is the use of gene therapy to treat haemophilia

2 Kill cells that cause cancers and

3 Counter the effects of exogenous genes that come from viruses

So far, gene therapy has **not** been found to be particularly useful for the treatment of bacterial or parasitic infections, although the approach may become feasible through the modification of patient genes to enhance antibacterial or antiparasitic effects.

If one considers the structure of DNA, which Watson and Crick determined, there is a very important feature of this molecule, which has a bearing on the feasibility of gene therapy. This picture is taken from the follow up Nature article that was published on the 30th May 1953. The important feature is that the DNA has a backbone that comprises sugar and phosphates which alternate with each other. The phosphate is negatively charged as DNA is an acid. The membranes that surround cells are made up mostly of fats, which provide a barrier to the entry of nucleic acids to cells. Crossing of a cell membrane by a therapeutic nucleic acid therefore does not occur naturally and there is a need for a carrier to deliver the gene therapy effecter to the site where it is intended to have its action. The principle underlying this problem is much along the lines of the way in which olive oil and balsamic vinegar separate and do not mix. Olive oil is a fat, much like the constituents of a cell membrane, and balsamic vinegar comprises acetic acid amongst other things and is analogous to DNA. Another factor that makes getting DNA into a cell difficult is that nucleic acids are very large, unlike most small molecule drugs that we are familiar with such as penicillin, and

this also makes the process of delivering the nucleic acid therapy across a cell membrane difficult.

There is a variety of different carriers that has been used in gene therapy to transport DNA or RNA to the inside of cells. However it is true to say that this remains the single most important obstacle to the realisation of the full potential of gene therapy. There are essentially three types of vectors,

1 engineered viruses
2 chemically synthesised carriers
3 and something in between which borrows from principles of viral vectors and synthetic vectors.

Each of these methods has advantages and disadvantages, but it is generally true to say that not all have properties that are suited to all applications. Synthetic carriers are useful for introducing RNA into readily accessible tissues, but do not achieve delivery of DNA for production of proteins in the body. Engineered viral vectors are generally a lot more efficient but have drawbacks of being expensive to propagate and prone to complicating toxic effects. The parent viral vectors have the benefit of many years of evolution to optimize the delivery of their genetic material and it is this property that is harnessed by gene therapy researchers. I recently attended the American Society of Gene and Cell Therapy where an interesting presentation from a Japanese researcher emphasized the advantage of using viruses for delivering genes to target cells. He described a comparison in cell culture between the best manmade vector and a virus particle for delivering DNA. When all else was corrected, he found that the viruses were about 4,000 times more efficient. This is probably an underestimate as the experiments were carried out in cultured cells and not in a living animal.

Our research interest has been in attempting to develop the use of gene silencing techniques for inhibition of virus infection. We have tried the approach with varying degrees of success against different viruses, and I will only describe one set of data that employs viral vectors to inhibit HBV infection. There are many researchers in the world using similar approaches, and the interest in gene silencing as a therapy was initiated after the discovery of the pathway in 1998 by Andrew Fire and Craig Mello. This discovery was very significant

for biology in general and gene therapy in particular, and this significance was recognized by awarding the Nobel prize for Physiology or Medicine to Fire and Mello jointly in 2006. The Nobel prize awarding committee cited 5 important factors that led them to their decision to award the prize and these are that the pathway provides:

1. Protection from viral replication

2. Silencing of mobile genetic elements

3. Regulation of protein synthesis

4. Transcription modulation

5. **Potential for therapy**

The potential for therapy was quickly picked up by the pharmaceutical industry as it was recognized that RNA potentially addresses a difficulty that many pharma companies are currently facing. These factors are firstly that there are only a small number of disease-causing genes that may be targeted using conventional drugs and many diseases cannot be treated using this conventional therapeutic approach. A huge advantage potentially of RNAi is that powerful and specific gene silencing is enabled and the process of developing something that can silence an intended target gene can be carried out rapidly to reach a stage of proving principle of efficacy quite rapidly.

Naturally in the cell, the pathway of RNA interference is initiated by the production of RNA from a DNA template within the genome. The prototype natural pathway is for the processing and silencing of genes by the micro RNAs. At the last count there were 1024 micro RNAs in the human genome, and these are very important for the regulation of gene expression, and the effect is mainly carried out by a silencing inhibitory mechanism. A characteristic feature of the RNA is that the sequences fold back on themselves to form hairpin-like structures. Sometimes there may be a few hairpins within the so-called primary microRNA sequence. The primary micro RNA is then chopped up to release individual

hairpins, which are exported from the nucleus to the cytoplasm. It is here that the precursor microRNA is trimmed by a dicing enzyme to remove the loop and short unpaired pieces of RNA and a short double stranded piece of RNA remains, which is the mature micro RNA. One of the strands of this short double stranded RNA is then selected and incorporated into a complex of proteins that carries out the gene inhibitory function by forming complementary pairing with a target messenger RNA and then effecting degradation of the target sequence. The interest that we have in this pathway is that it is possible to introduce mimics of natural micro RNA intermediates, which can be engineered to produce gene silencers according to any intended design. In our case we are interested in generating artificial engineered sequences that are capable of inhibiting hepatitis B virus gene expression. The theory is that the gene sequences of HBV are specific to the virus and not found in the human and therefore specificity can be achieved to knock down the expression of the virus gene specifically.

This infection with HBV is an important global health problem. It is estimated that 387 million people are persistently infected with the virus and the areas where infection is commonest are in sub Saharan Africa and in east and south east Asia. When infection occurs in childhood, it frequently persists and this form of the infection is what leads very often to the serious complications of cirrhosis and liver cancer. The risk for the life-threatening complications is very high and about 25% of people who are carries will develop the complications. The cancer is one of the most aggressive malignancies and the prognosis is very poor. However not all is bad news and the reason is that there is an effective vaccine against HBV, which became compulsory in South Africa in 1995. Nevertheless, the number of people already carrying the virus remains very high and vaccination will not help them. It is for this reason that we have developed an interest in developing new methods of treating HBV infection and why we have advanced methods that employ RNA interference to do this. The results to date are promising and the main focus of our research is the development of the candidate HBV gene silencers to a stage where they may be used as a therapy in patients.

As mentioned before, it is possible to devise a strategy to inhibit HBV gene expression that is based on a rational approach, and this is the great strength of gene therapy. Some approaches are better than others and some rules have been worked out to help in the design. The method that we used was based on some simple principles, which were then tested empirically to arrive at the best antiviral gene silencers. The genetic material of HBV

comprises a circular arrangement of DNA and encodes 4 genes that overlap with each other. We chose a region that was very conserved on HBV isolates. This means that the targets were present in viruses from different parts of the world and the other guiding principle that we used was to use target sites that were common to the different messenger RNAs that the virus produces. To cut a long story short, we made artificial intermediates of the RNAi pathway that formed hairpin structures that simulated intermediates of the RNAi pathway. These were then tested in cultured cells and the best were selected for further development. Essentially, these anti HBV sequences were artificial genes that formed RNA that mimicked the hairpin RNA intermediates.

As mentioned before, a critically important concern for advancing gene therapy to clinical application is the efficient and safe delivery of the DNA or RNA in question. This is the old olive oil and balsamic vinegar story. We have used a number of different approaches and one which has worked particularly well in our hands is the adenovirus vectors. These viruses are common causes of upper respiratory infections and many of us in this room have been infected with the virus at some stage of our lives. You may well be wondering why use a virus as a vector to treat liver diseases when the tissue that is naturally infected is the respiratory tract. That is a very good point, but a very interesting feature of the adenovirus is that when one injects it into the blood, say through a vein, it is latched on to by a blood clotting factor that naturally occurs in the blood, which is clotting factor 10 (X).This interaction between the virus and blood clotting factor 10 confers so-called liver tropism on the vector. This means that the engineered virus homes in and infects liver cells. Of course this is also where HBV infection is occurring, and is convenient when one wants to deliver something that will act against HBV.

Manipulating the adenovirus vector to incorporate the anti HBV sequences is a particularly interesting piece of molecular biology engineering and can be summarized in the following few slides. The genetic material of adenoviruses comprises double stranded DNA that has a linear straight line-type arrangement. The first step is to manipulate the purified adenovirus DNA to incorporate and anti HBV sequence. This can be done using methods of molecular biology that entail cutting and pasting of different pieces of DNA together. In addition to the anti HBV sequence, a marker or reporter gene is also included to enable us to track the virus and see which cells are being infected by the engineered virus. In our

experiments, we used a reporter gene which encodes a protein called green fluorescent protein or GFP. Interestingly, the gene was originally isolated from a jellyfish of the *Aequoria victoria* species. The third and very important feature of the engineered adenovirus is that it should be disabled to ensure that it does not pose any hazard to the environment. To do this, what is done is that some essential genes are removed from the virus to render them sterile. This means that the engineered viruses are capable of infecting cells but cannot them propagate themselves to complete their life cycles.

I have selected some data to show you that present the results of studies that we carried out on HBV mice. One of the difficulties of working with HBV infection is that there is no convenient small animal model of the infection. HBV does not naturally infect mice, and there is not a similar virus that infects mouse livers and it is necessary to manipulate the viruses so that HBV proliferates in the animals. These mice we received from collaborator in the US at Stanford university and have been extremely useful for our work on trying to advance treatment of HBV infection. Initially, to assess whether the engineered anti HBV adenovirus was reaching the liver, we measured the presence of green fluorescent protein in the liver. This slide shows a representative example, where it is clear that most of the cells in this slide fluoresce green when they are illuminated with light of a specific wavelength. We considered this enough for a therapeutic effect and proceeded to test anti HBV efficacy. We used many different measurements to verify that and antiviral effect was being achieved, but the one that is shown here, and which is a standard clinical measurement of HBV proliferation, is the assay of the envelope or surface protein in the blood. What can be seen from this graph is that the mice that received control adenovirus vectors, in other words with irrelevant hairpin intermediates, had no effect on the concentration of the surface protein in the blood. These are the three red and orange coloured lines of the graph. However when an anti HBV sequence was included, a profound decrease in the concentration of the marker of HBV replication was observed. These are the blue and purple lines of the graph. Please note that the Y axis scale is logarithmic, which appears to make the effect less dramatic.

In summary the results of this and other work that we have completed on using gene therapy to silence hepatitis B virus infection have demonstrated that the approach is feasible as it works reasonably well in a small animal model of the infection. The treatment approach is however not a standard one and developing a complex biological formulation to make a

drug is fraught with difficulties. The discovery of carriers that can be prepared inexpensively and which are safe and carry out their delivery task efficiently remains paramount.

The issues that need to be resolved for the delivery problem can be broken down as follows. Firstly, there is a need to get the gene silencer to enough cells to have a good therapeutic effect. We know that because of the charge and large size of DNA and RNA, this is difficult. The second issue that needs clear definition and resolution is that the delivery be to targeted to the affected cells, which for HBV infection is of course the liver. Thirdly, we know that HBV infection persists for a long time and can remain dormant then reactivate itself. There is therefore a need to ensure that delivery of the therapeutic nucleic acids is sustained to ensure that the virus is 'knocked out'. Because the gene therapy drug is complex and contains many components, the nature of the drug is complicated and not subject to the same rules as standard so-called small molecule drugs. The side effects are different as are the distribution in the body, immune response and dose regulation. Lastly of course the preparation of complex drugs is expensive and when one considers that there are as many people in the world who are infected with HBV, and the fact that most of these people are from resource strapped countries, this is critically important to address.

Dealing with these problems successfully will be a large task, but it is also what will determine whether the approach will succeed or fail. I remain optimistic about gene therapy as a very powerful approach, mainly because it is amenable to rational design and therefore has enormous applicability. My current research focus is therefore is moving away from optimizing HBV gene silencers, which we are now confident work well, and it is now aimed at addressing issues relating to delivery of anti HBV nucleic acids to infected liver cells. Large scale inexpensive preparation of suitable vectors will be key to the success, and valuable lessons can still be learned from viruses. We are currently using approaches that combine adaptations of virus molecular biology with methods that are amenable to large scale preparation of vectors. That is through utilizing chemical synthesis procedures and also harnessing that wonderful workhorse of molecular biology, which is the bacterium, *E. coli,* our aim is to propagate safe vectors that can be made on a large scale. Viruses have essentially evolved mechanisms of delivering nucleic acids to cells which addresses the prime difficulty of gene therapy. If one considers the early stages of a virus infection, essentially the initial steps are the attachment of a viral particle to the surface of a cell, and this is followed

by release of the viral genetic material, which then makes its way into the infected cell to code for the propagation of progeny virus particles. What I am proposing is that it is not completely intact viral particles that should be utilized, as was done in the experiments that I described with the adenovirus vector, rather it is to assemble part of a virus with the desired properties say of carrying a DNA payload in a bacterium and combining this with synthetic chemistry procedures to make a safe intact vector particle. There is of course a need to be selective in harnessing virus properties, and this aspect will need to be tested rigorously.

Many of you are no doubt wondering what it is about the subject that I have been speaking about that makes it appealing. It is of course something that I spend most of my time working on and the subject has a particularly significant personal appeal. The quote from Thomas Cech articulates the essential points that concern me rather well. Thiomas Cech received the Nobel prize for chemistry in 1989, together with Sydney Altman, for his discovery of the enzymatic properties of RNA. This was a particularly interesting finding because the implication is that RNA was the primordial molecule of life. The quote presented here is essentially a comparison between scientific and artistic approaches to achievement.

**…. Science is knowing nature. This implies that there is a fixed set of facts that everyone wants to know. ….. I suspect that artists are not striving for commonality, but rather, that the content as well as the form of their work is unique.**

Usually one uses the term discovery to describe the process of scientific research achievement. Conversely, for artists, it is creation that is what artists essentially do. As an example, Watson and Crick applied their considerable analytical and interpretative skills to deduce the double helical structure of DNA from the images that they used from X-ray crystallography. They were able to take the images that were formed from bombarding crystals of DNA with X-rays and derive the double helix. As you can see from the type of image that they utilized for their interpretation, there was a certain amount of abstract reasoning that was required to arrive at the double helical structure. It is however important to point out that it is highly likely that the structure would have been worked out later if they had not been successful. There were a lot of eminent and capable scientists working on the problem, which would have been solved eventually. Also, the structure has always been present in life as we know it and will always be there. Watson and Crick unraveled something

that was very fundamental to biology and as mentioned before was probably the most important discovery of the 20th century. Of course they did not create DNA. Creation of things is the realm of the artist. Picasso's Guernica masterpiece is an example of this. The painting was prompted by an event that occurred in 1937, when the German Luftwaffe attacked the Spanish Basque town of Guernica and killed many civilians. The painting became a reminder of the tragedies of war and embodiment of peace. Regardless, the important point is that there was not a Guernica painting waiting to be discovered. Rather it was Picasso who created it as a result of his interpretations of the tragedies of war. He may have had some influences, such as from Goya, but essentially his masterpiece was a synthesis of his own ideas that are represented in his painting. What has this got to do with gene therapy you are no doubt asking yourselves. For me, gene therapy is a line of investigation that straddles science and the creativity of art. There are some very important scientific rules and laws which have a bearing on the field, but the ideal gene therapies do not exist as fundamental laws of nature. When the ideal gene therapy for HBV is developed, it will be based on creatively applying fundamental laws to form a useful drug. In a way this is similar to engineering disciplines and architecture.

To sum up, gene therapy is an important field and there is enthusiasm amongst many researchers throughout the work for its potential. Although therapies are not yet widely applicable, it is true to say that scientists remain enthusiastic about the potential of the technology. Progress has been rapid, new technologies are continually being developed and successive waves of innovation will be required to contribute to the eventual success of the approach. Related technologies will be important too. For example the rapid advances in sequencing technologies will have a bearing on the applications of gene therapy. The genetic basis of diseases that have been difficult to understand will unravel and gene therapy may in the future be applied to treating multifactorial diseases. The slide here illustrates the breathtaking advances in the DNA sequencing technology that have taken place during the past 10 years. This information was taken from Nature in April last year and may well be out of date. The important piece of information on one of the graphs indicates that it cost about 10,000 US dollars in 2000 to sequence a billion base pairs, and in 2010, the cost had plummeted to 1 US dollar. Along with this drop in price, there has also been a huge 50,000 fold increase in the speed of DNA sequencing.

Another important point about the prospects of gene therapy is perhaps a commercial consideration. It is known that the number of blockbuster drugs that will lose their patent exclusivity will be more than the number of drugs that will be approved. It is very difficult and expensive for drug companies to take drugs from stages of early development to clinical use and along the way there are many failures. The feature of gene therapy that is amenable to rational design of drugs is very powerful and will make this type of therapy all the more important in years to come. Related to this, it is critically important that South Africa does not rely on developments made elsewhere in the world. Rather South Africa should participate fully in this exciting technology.

Finally, I would like to mention some of the many people who have influenced me in developing the ideas that I have presented tonight, and some of them, not all are listed in the last 2 slides. Firstly, my PhD supervisor Professor Wynfried Fitschen, who was a very rigorous and careful scientist. He also impressed me with the notion that counterintuitive explanations for biological process should always be considered. The second person who was very helpful to me was Professor Michael Kew. He introduced me to the field of liver diseases and hepatitis B virus in a way that was most interesting and prompted my to focus most of my career on this topic. I have collaborated with many researchers from South Africa and abroad and they have also contributed to my own development. These have included the French scientists Nicolas Ferry and Christain Bréchot. The US HBV molecular biologists Bill Robinson and Pat Marion from Stanford university and Mark Feitelson from Thomas Jefferson University in Philadelphia.

The team with whom I work have always shown impressive youthful enthusiasm and this has made for a wonderful environment for me to work in. It is very rewarding that the enjoyment of the work has been shown has translated into good productivity. I would like to make special mention two current members of the team who have been particularly important to the recent scientific achievements. They are Marc Weinberg, who was my first PhD student and is now an associate professor in the unit and developing his own research programme. The other is another past PhD student of mine. He is the quiet, talented and ruthlessly focused Abdullah Ely.