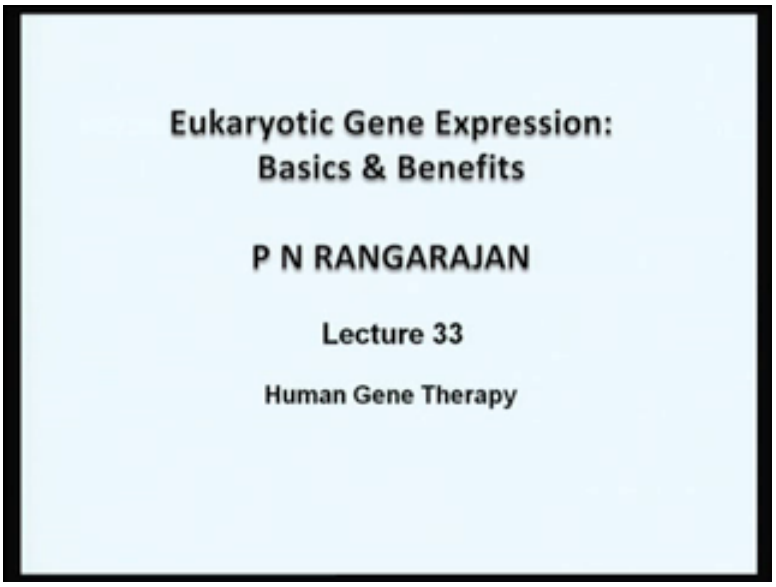
Eukaryotic Gene Expression
Prof. P N Rangarajan
Department of Biochemistry
Indian Institute Of science, Bangalore

Lecture No. #33
Human Gene Therapy

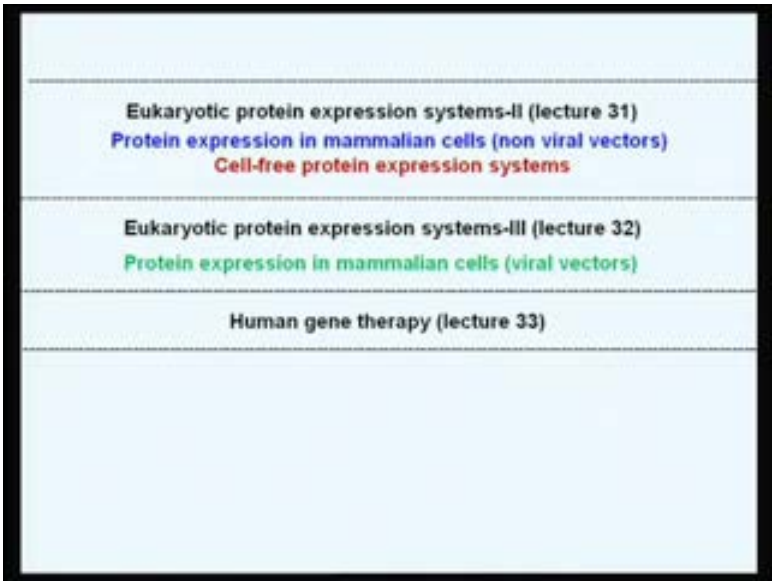
Welcome to this lecture series on eukaryotic gene expression basics and benefits.

(Refer Slide Time: 00:26)



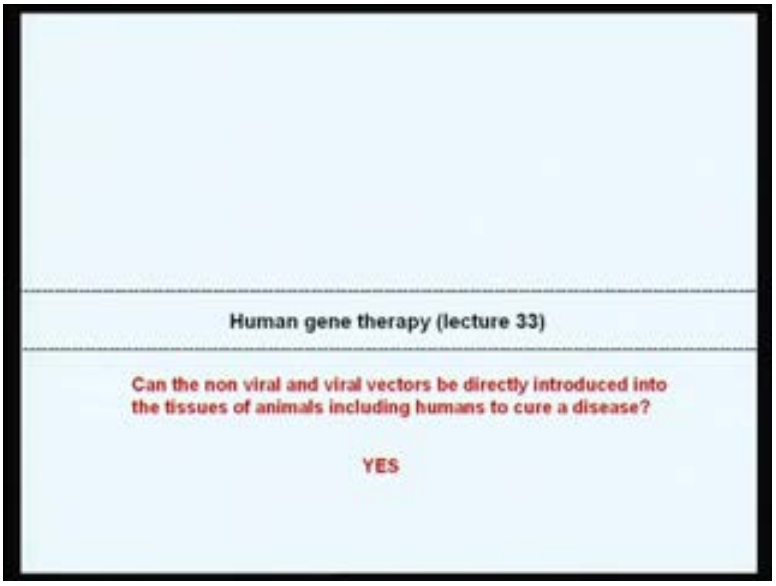
Today we are going to discuss about a very fascinating area of biology and medicine, known as the human gene therapy, the last two classes we have spent considerable amount of time discussing about introducing genes into mammalian cells using viral and non-viral vectors, this is what shown here.

(Refer Slide Time: 00:43)



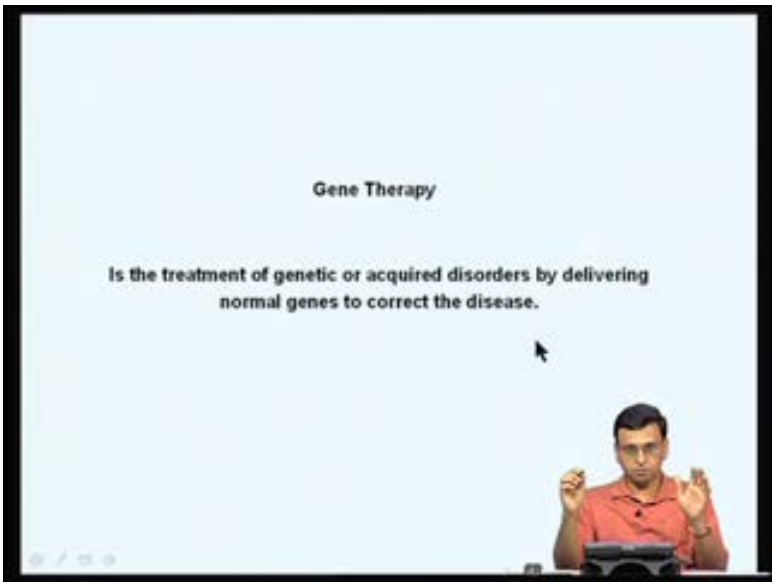
We spent considerable amount of time discussing, how we can introduce genes and express proteins in mammali San cells using non-viral vectors as well as viral vectors. Now once the technologies are introducing genes into mammalian cells and expressing them successfully using viral or non-viral vectors for developed, then people started asking the question, instead of introducing genes and mammalian cells, expressing their proteins and then purifying the proteins, and then using for therapeutic purposes, why cannot you use this non-viral and viral vectors, and directly introduce this vectors into the humans itself. So, that these vectors would go with specific tissues, transfect specific cells and the gene will be expressed, and protein will be made ride inside our body, so do not want to express genes, either in using this expression system or e coli expression systems or insect or mammalian systems, then purify the protein and then give it as a therapeutic, instead use this mammalian expression systems either viral or non-viral, directly inject this vectors into the humans, and see whether the protein can be made right inside our body, this methodology of using viral or non-viral vectors to introduce genes into the body with the objective of curing the disease has now come to be known as gene therapy, it is a very exciting area of research. So, today we will focus on human gene therapy.

(Refer Slide Time: 02:12)



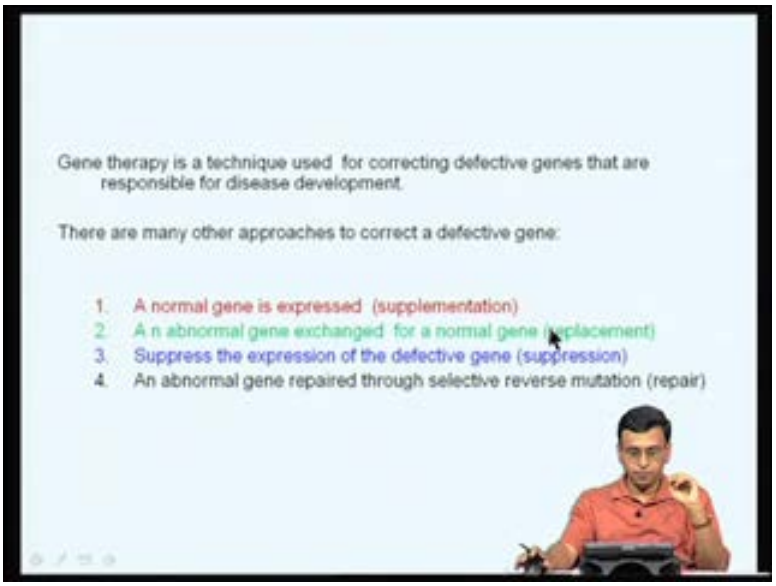
So, the question we are asking is, can the non-viral and viral vectors which we have been discussing about using the expressing genes and mammalian cells in culture, can there be a vectors including that particular gene directly into the humans, so that the therapeutic protein will be made inside the humans and the disease can be cured. So, can the non-viral viral vectors be directly introduced in tissues of animals in to cure a disease, the answer is yes, so let us now see how we can do this?

(Refer Slide Time: 02:43)



So, the definition of gene therapy basically is, the treatment of genetic or acquired disorders that delivering the normal genes to correct that particular disease, this is what is the very simple definition of gene therapy.

(Refer Slide Time: 02:57)



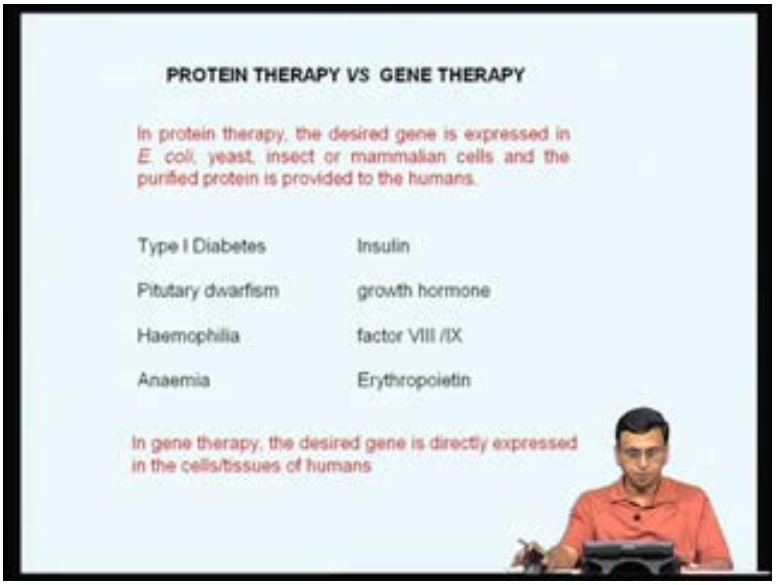
The gene therapy can be used for correcting defective genes that are responsible for disease development, that is main ways of doing this, in the normal way usually the most of the gene therapy protocols known a various genetic defect, there is a gene which is mutated or it is deleted as a result its specific protein is not made, what you now do is, you take the correct gene or the normal gene for that particular protein, put them in a nyle viral vector non-viral vector and introduce this into the human. So, this is where we are basically supplementing the normal gene to the human beings, but the defective gene is still there, but the function is restored by expressing the normal genes, so therapeutic protein will be made. So, a normal gene is a special basically a supplest a supplementing the individual with a normal genes, so that the normal protein can be made, this is most of the gene therapy protocols is ended this particular kind of a approach, but a much more idea approached should have been to actually exchange or replaced the normal, the defective gene with a normal gene. By actually what is called as a homologous recombination.

So, you can actually replace a defective gene which is present in the chromosome with a normal gene, so that the defective chromosomal copy of the gene is removed and a

normal gene is expressed. So this is the ultimate objective of gene therapy, but we still have not reached this level of sophistication to aim at gene replacement therapy. The other thing that people are trying out is so what is called as suppressed expression of a defective gene, usually in disease like cancer when you know the cancer is, because of expression of an anco genes over expression of anco genes using molecules, such as antisense R N A or what are called as S I R N A's or M I R N A's which we discussed in mutated classes, can we actually block the expression of a endogenous gene, so this is called as a gene suppression.

A much more challenging aspect of gene therapy is what is called as a repair, why in abnormal gene if a particular gene as a mutation or deletion, can you remove can repair this endogenous gene by actually introducing the current gene, so that the endogenous homologous recombination or a gene repair brain repair pathway, now through homologous recombination, replaces the endogenous gene with a replacement gene is called as a endogenous repair. So, number of approaches are possible when we thought think about introducing the gene to correct a particular genetic disorder, but the most common approach that is being practiced or being tried out in various clinical trials, is to see if a defect is there in a particular gene as a result a specific protein is not being made into such individuals can we introduce a normal gene, through a non-viral vector or a viral vector, so there that particular protein can be made and their patients can be cure of a particular disease, this is what is where we are going to discuss now.

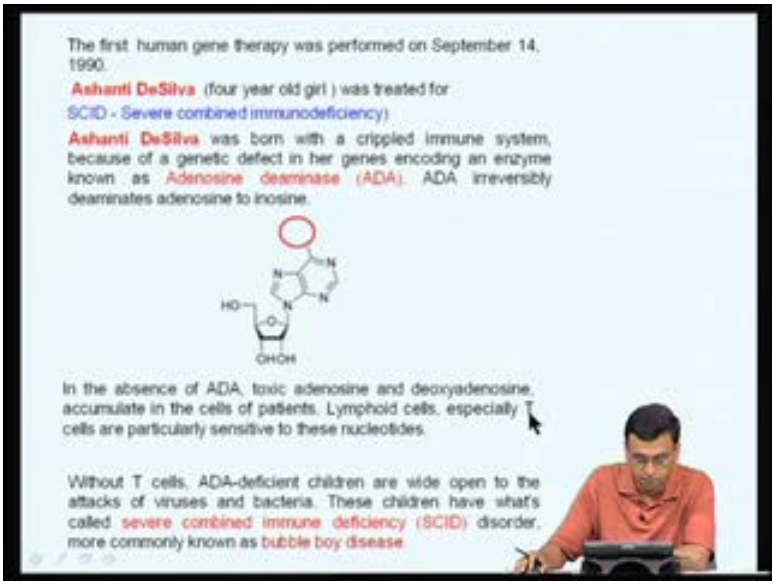
(Refer Slide Time: 05:50)



The gene therapy is kind of an extension of a protein therapy which have been discussing, because the last five to ten classes we have basically talking about various expression systems, how we are using the e coli or e yeast or insect or mammalian expression systems, to make a number of proteins of therapeutic or diagnostic importance in large amounts, purify them and used it as a diagnostic or therapeutic purposes, this is what as protein therapy. For example if you are person suffering from diabetes, here is an insulin, so you take the insulin gene, put in e coli system or yeast systems or a mammalian system, expressed in large amounts, purify insulin and delete for treatment of diabetes, this is what is being called as a protein therapy.

Similarly, if a person has dwarfism, because of a defective growth hormone gene, you again take produce growth hormone in large amount on a expression system, either e coli or a eukaryotic system and then purify this and give it as a therapeutic. Similarly, if a person is suffering from hemophilia you can take the factor gene express in appropriate expression system, purify this protein and give it as a therapeutic and so on and so forth, for anemia you can express erythropoietin and so on and so forth. This is a typical protein therapy in case it has been very successful and a number of therapeutic proteins made in a using recombinant D N A technology, using a number of heterologous expression systems are quite today available in the market, but a question now where asking is that, instead of making this proteins in either e coli or yeast or insect or mammalian cells, why cannot we are take this gene, and directly introduce this gene into the human cells or tissues, so that the protein is made right inside our body, and we can be benefited, that is the difference between a protein therapy versus gene therapy.

(Refer Slide Time: 07:28)

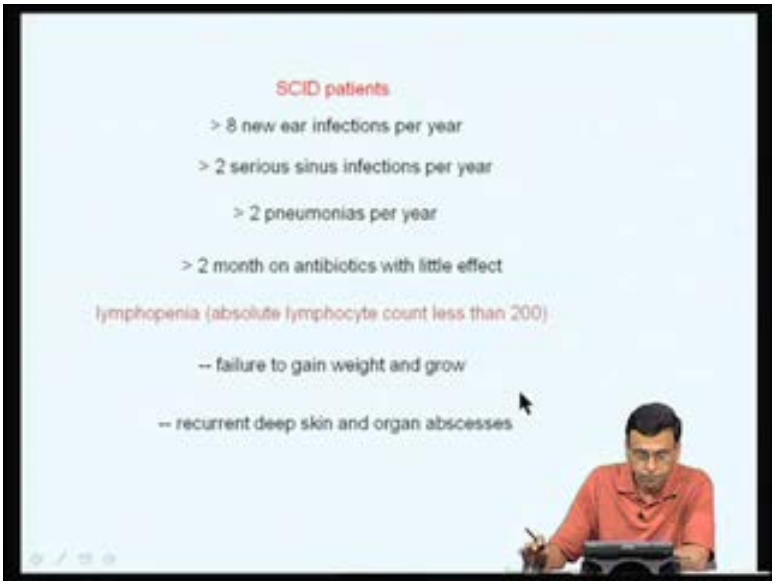


The first human gene therapy was actually performed in September 14, 1990. So, it is about two decades old, the human gene therapy is about two decades old, this was actually done on a girl called Ashanti D'silva, she is a 4 year girl she was suffering from a genetic disorder called scid, means severe combined immunodeficiency, now what is this disease, she was actually born with a crippled immune system, have normal immune system. Therefore, we can fight in a disease, that is viral or bacterial or parasitic, our immune system can fight and we can get cured, we can protect ourselves from a number of pathogens, but in the case of Ashanti D'silva there was a problem in her immune system, therefore immune system is not well developed, that is because her body there was a defective gene coding for an enzyme called adenosine deaminase, abbreviated as A D A. Now, what is the function of A D A, A D A reversibly irreversibly deaminates adenosine to inosine, this is what is adenosine and what it does is, it removes this amino group, so deamination of adenosine results in the formation of inosine, and is a very important step in puring metabolism.

Now, what happens if this enzyme is not there, if the enzyme is not there adenosine deoxy adenosine accumulate in the cells of this patients, and as a result especially in the case of lymphoid cells, immature lymphoid cells especially t cells they are very sensitive to these toxic levels of deoxyadenosine adenosine, therefore they die. So, these individuals will not have functional t cells, as you know t cells and these cells are very important part components of immune system, and if you do not have t cells you'll not

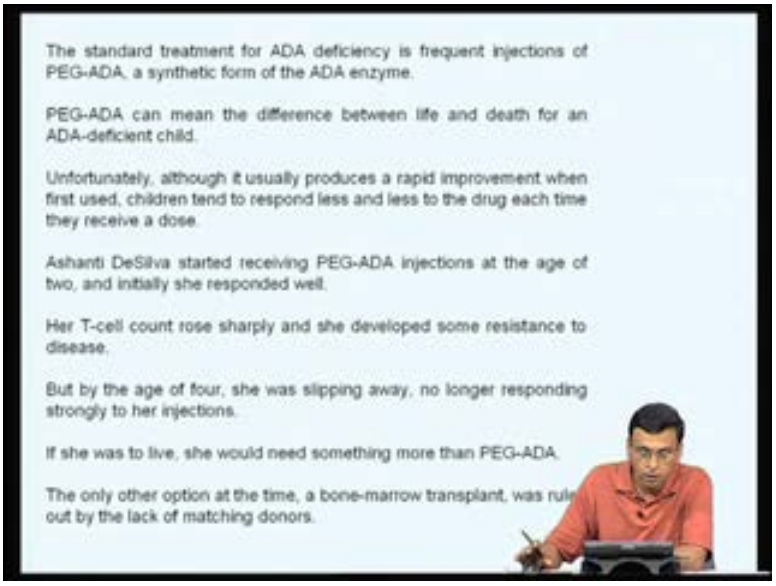
have cell mediating in responses, or even t helper responses, and as a result you cannot attack pathogens, you cannot defend pathogens. Therefore, without t cells these A D A deficient children or wide open to attacks of viruses and bacteria, and these children's, therefore have this severe combined immune deficiency disorders and this also known as a bubble boy disease, because these people all has to be in a sterile atmosphere, because they are easily susceptible for a number of diseases.

(Refer Slide Time: 09:41)



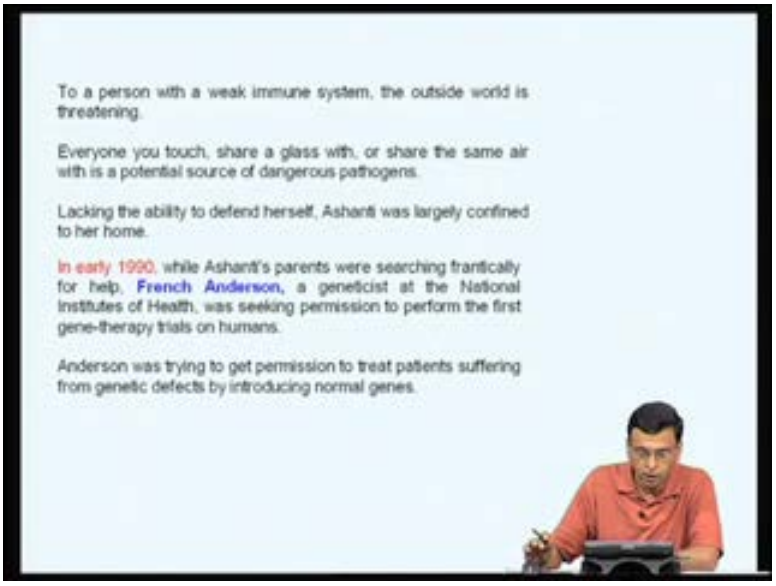
For example, many common diseases that you see in the case of this scip patients is, they have eight new ear infections per year, they have serious sinus infections, pneumonias, two month they have to be antibiotics all the time, because they are highly, they cannot fight many common cold and common bacteria which we normally. Fight with our immune system, they cannot do it so they have to depend on antibiotics, they have a very low count or low lymphocytes counts less than two hundreds, this called as clinically called as lymphopenia, they fail to gain weight, they do not grow very well and they have lot of deep skin and organ abscesses. So, a number of problems, because of this one enzyme is not made in our cell in our body we see the problems.

(Refer Slide Time: 10:25)



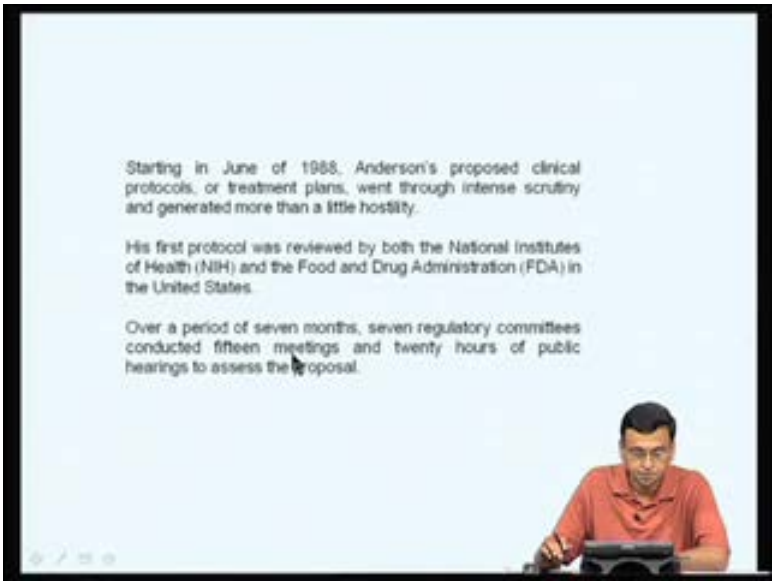
The standard treatment in the 1990's for this kind of a scip patients is, they have to frequently take injections of adenosine D M N A, conjugated with another molecule called polyethylene glycol called PEG-ADA, what is this A D A enzyme can be either purified from animals or it can be from a recombinant source, but whatever it is the A D A enzyme as to be conjugated with polyethylene glycol and they have to frequently take this peg A D A, so that the this enzymes now can supplement the defect. So, they have to take, the problem is that as they keep taking this peg A D A injections after period of time, they stop responding to this peg A D A, because that means they become they develop resistance, the peg A D A does not work as well as it work in the initial periods. Therefore, by the age of 4 this girl small little girl Ashanti D'silva was no longer responding to this peg A D A injections, so t-cell counts are dropping very badly, she was becoming very susceptible to a number of infections. So she has to be confined to home and as to be taken care take, extraordinary care as to be given to make sure that she does not catch any infections, the only available alternative at that time is do what is called as a bone marrow transplantation, for at that time, because of non available matching donors they could not do a bone marrow transplantation.

(Refer Slide Time: 11:52)



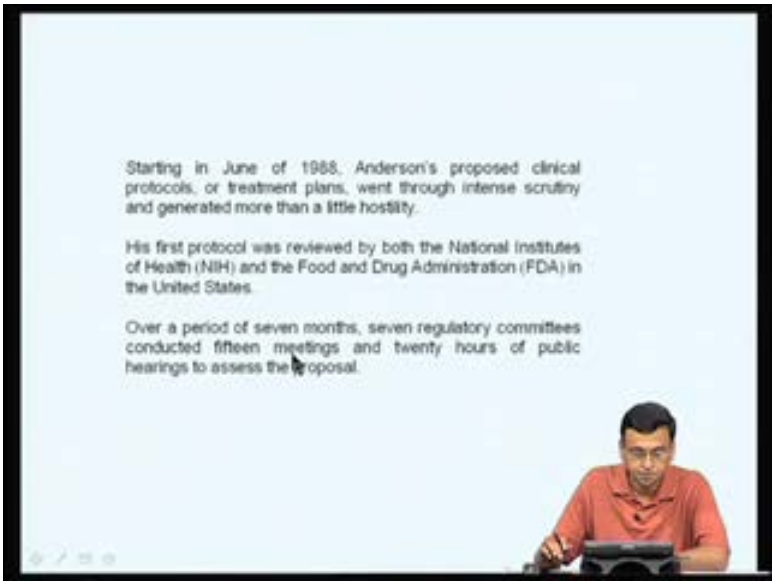
So overall or normal, because we do not bother about many of this common pathogen, because our immunes system take care it, but if a person has a weak in immune system, you can get infected very easily, even a touch by someone else or if you share a glass or even share hand with air, all of them are filled with pathogens and you can catch infections very easily, so Ashanthi was largely confined to our home, so they are desperately looking her parents were desperately looking to see, how we can keep her alive, what kind of a treatment can be given to her. So, on this time we are talking about 1990's. Now, this time a geneticist called French Anderson was actually approaching the national institutes of health to get permission to do what are called as human clinical trials, wherein he wants to introduce genes into humans in the A B B N of correcting genetic defects, he was trying to get permission to treat patients suffering from genetic defects by introducing normal genes.

(Refer Slide Time: 12:53)



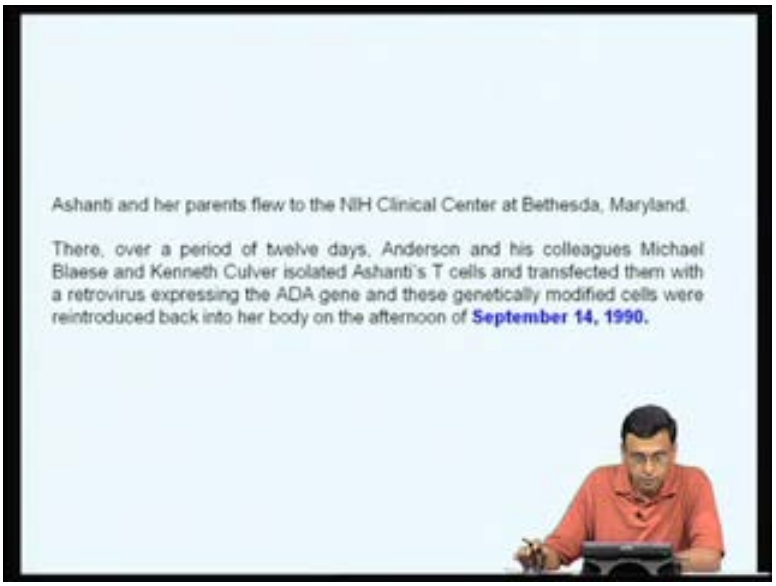
To be human trails especially a new form of, because nobody has done this before, people I think inject transfecting cell lines with the organs of things that, peoples are actually testing this kinds of the viral and non-viral animal models, and so that these things work, but to inject this kinds of a viral vectors or non-viral vector direct into humans was not heard off, so a very strict and very complicated regulatory procedure as to be followed, before he was given permission to do this kind of a methodologies in human beings. So, starting from June of 1998 Anderson proposal was reviewed by what is called as a National Institutes of Health, and the watch drug in the U S called as U S Food and Drug Administration, and after a series of about the consultations and reviews meetings over a period of seven months, including twenty four hours of public hearings to assess his proposal.

(Refer Slide Time: 13:48)



Finally, he was given permission in the earlier 1990's, to do a human clinical trial to treat a patients suffering from genetic disorders, especially the severe combined immune efficiency syndrome. So, Ashanthi was the right candidate, because the parents were actually looking for some kind of a permanent cure.

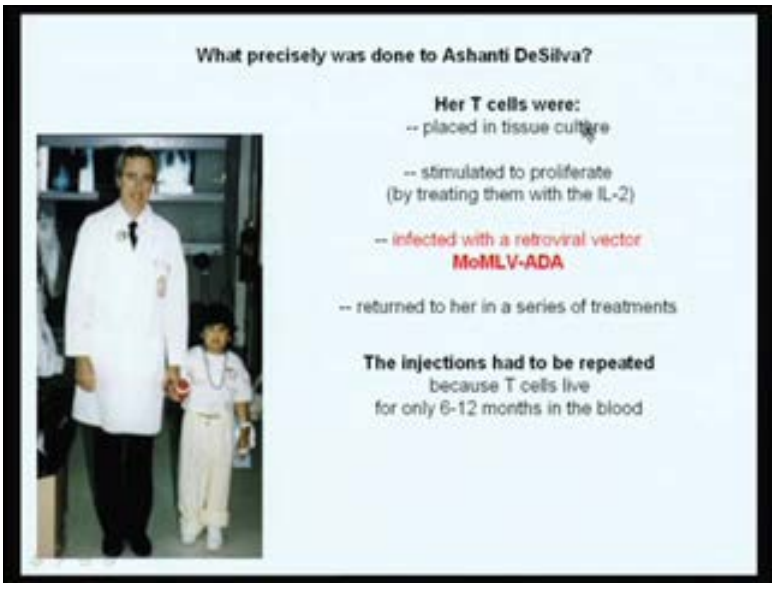
(Refer Slide Time: 14:08)



So, Ashanthi and her parents flew to in a N I H Clinical Center at Bethosda Maryland United States, and over a period of twelve days Anderson and his colleagues Micheal Biaese and Kenneth Culver isolated Ashanthi's t cells, and transfected them with a

retrovirus expressing the A D A gene, and these genetically modified cells were introduced back into our body in the afternoon of September 14.1990. So September 14.1990 was a very important landmark in the history of human gene therapy, because that is the time genetically modified cells where introduced to back into human patient by Anderson and his colleagues, the first example of a human gene therapy was conducted. So, we have all studied about the retroviral mediated gene transfer adenoviral mediate gene transfer and so on and so forth in the last class, so basically what you do you, you take the gene put it in retroviral vector and take this retroviral vector, and put it in a packaging cell lines and a packaging cells now add the viral proteins, and the replication defect will recombinant retrovirus now will emerge out of this packaging cells, these recombinant retroviruses carrying the normal A D A gene, is now used to transfect this cells of the patient, and then these cells which now contains stable integrated retrovirus, and from the retroviral genome the R N A will be made, and the protein will be expressed, and this protein will now be normal, patients should be normal. So, these kinds of a engineered cells is now put back into the body of the patient, this is basically the procedure that was done.

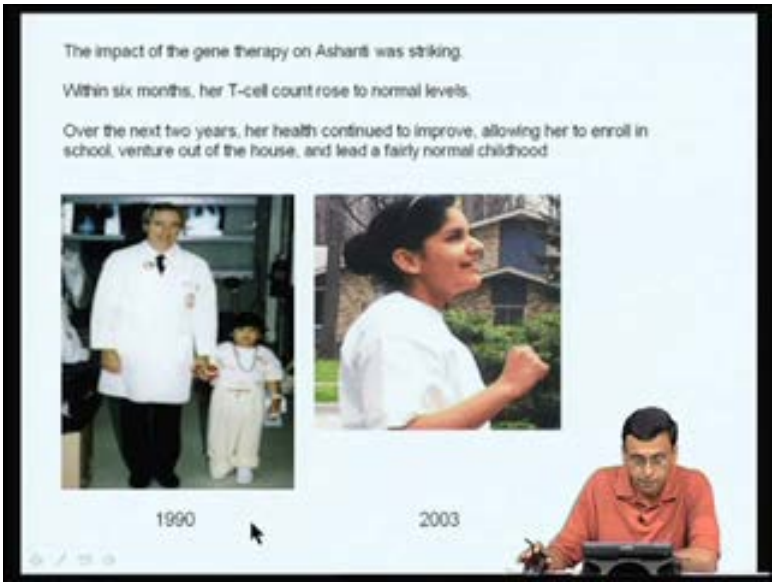
(Refer Slide Time: 15:39)



So her t cells were placed in tissue culture, they are stimulated to proliferate by using cytokines like I L 12 or inter looking 12, so once the t cells are proliferate, they were infected with the retroviral vector, remember retroviruses will only infect device in cells, therefore you have to stimulate proliferatno t cells, using lymphocytes or cytokines like

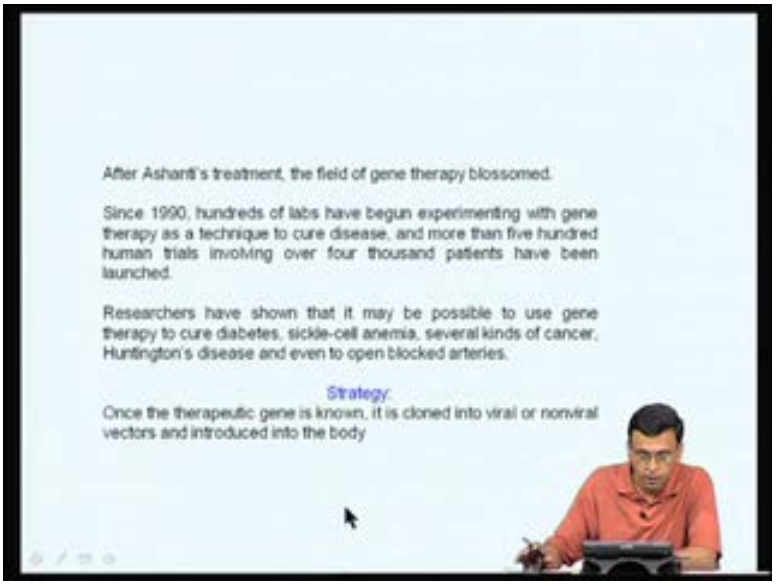
inter leukein twelve, and once the t cells start proliferating, you have a replication defective recombinant retrovirus expressing adenosine deaminase, now the retrovirus will go integrated with a genome of this dividing cells, and the gene will be expressed. And now once you take this cell which are now expressed, the transfected with the retrovirus, and put them back into your body, this was they were done for Ashanti D'silva, this is doctor French Andeson, and this is the four year little girl, who was the first human candidate for undergoing gene therapy.

(Refer Slide Time: 16:30)



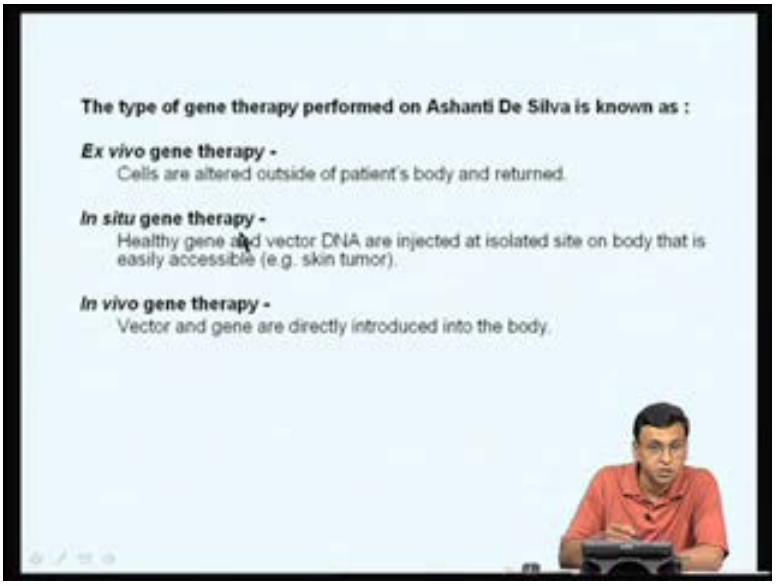
The impact of gene therapy and Ashanthi was welcome with the lot of surprise and lot of anticipation, and within six months her t cells count goes to normal levels, off course she was also receiving the normal supplementation of A D A pack, because nobody could derive the normal injections, because nobody knew how effective her gene therapy would be, but over the next two year, her health continuity to improve, allowing her to enroll in school, venture out of the house, and lead a fairly normal childhood, so this is the picture that was taken in 1990 when she was performed a gene therapy, when she was in four years old, this is a picture taken in 2003, and you can see over thirteen year old girl become now looks normal, so this was the first successful example of a human gene therapy performed.

(Refer Slide Time: 17:15)



So following this successful human gene therapy using Ashanti D'silva, hundreds of labs began experimenting with gene therapy as a technique to cure disease, and more than five hundred human trials involving over four thousand patients have been launched, over the last twenty years. Researchers have shown that it may be possible to use gene therapy not only to cure genetic disorders, like hemophilia scid and so on and so forth. They can also be used for the treatment of number of acquired disorders, like diabetes, sickle cell anemia, cancer, huntington disease, and even acquired disorders like coronay artery, they have blocked arteries, they can be reopen using appropriate either by expressing appropriate gene therapy techniques. So, the strategy has been, once the therapeutic gene that you have to introduce is known, you clear about what kind of gene you have to introduce what kind of protein as to be made, you take that gene cloned into viral or a non-viral vector and then introduced into the body, this is been the basic strategy for doing all this gene therapy technical trials.

(Refer Slide Time: 18:21)

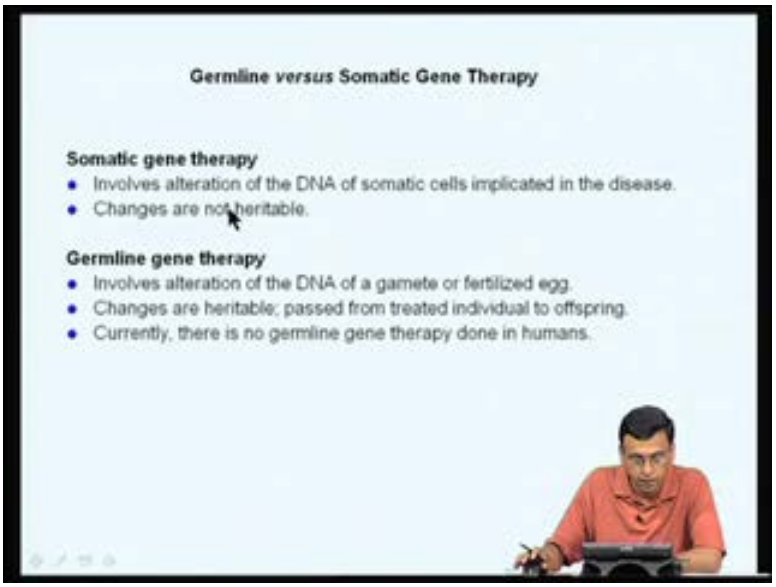


Now, the type of gene therapy that was performed on Ashanti D'silva is known ex vivo gene therapy, that is what you have basically is done is you have taken the cells from the patient's body, culture them in vitro, transfected them with your gene of your interest, either using viral or a non-viral vector, and then these cells which have now contain your transgene of your interest now and you take them out and then put them back into your body, this is called as a ex vivo gene therapy where cells are altered outside that patient's body and then returned. Now, there is also involved a direct methods what are called as in situ gene therapy and in vivo gene therapy, in the situ gene therapy, a healthy gene encoded by a vector is injected at the isolated site in a body that is easily accessible, in situ means you confined to very small region.

Suppose, for example you have developed a tumor, you can actually take a virus encoding, a cytokine gene or a virus encoding, the allow antigens and inject this vector directly into a tumor, so that you are only injecting the gene or the vector a very low class regions inside the body, so this is known as the in situ gene therapy. A much more general approach are common method is what is called as a in vivo gene therapy, where the vector in gene usually introduces systematically into the blood, so that the vector goes and then infects a number of organs or if there are some specific receptors it goes to targeted specific organ, is a much more generalized and more direct form of gene therapy, where the vector and gene are directly introduced into the body usually using a

systemic approach. So, these are the three types of gene therapy usually performed, ex vivo gene therapy, in situ gene therapy and in vivo gene therapy.

(Refer Slide Time: 20:08)

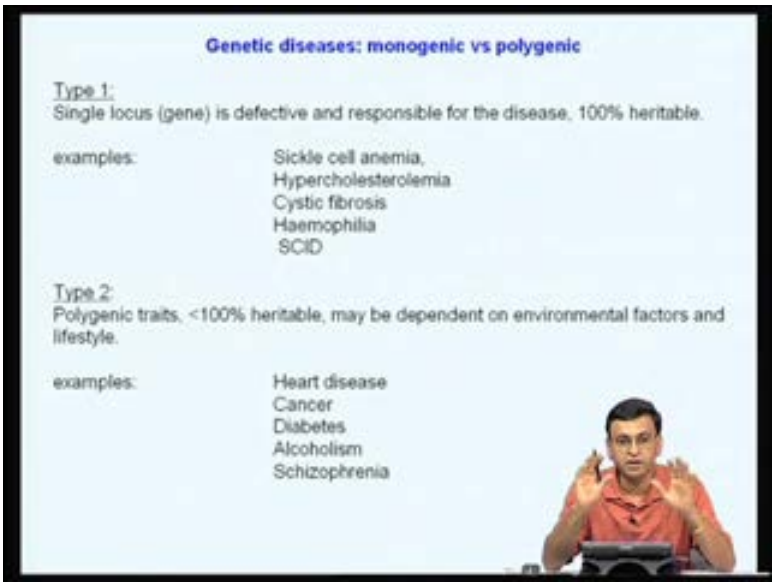


There also a very two major distinctions, the gene therapy again that was performed on Ashanthi is what is known as a somatic gene therapy, where yet actually taken the blood cells or the white blood cells or the lymphocytes forms her body, introduce your gene using retroviral vectors and put them above. So, as long as introduced your genes or the vectors into the somatic cells of body, it is called as the somatic gene therapy, where it involves alteration of the D N A of somatic cells implicated in the disease. So, in such cases the change of that you introduced are not heritable, so the retroviral gene therapy be that one can only cure the patient who has received, the children born to them still have the same genetic disorder, so only the person is cured, because your introduced gene is going to somatic cells, the germ lines, the germ cells still carry the defective gene, therefore their offspring's genes still susceptible to the genetic disease.

A much more ideal form of a gene therapy would be, to ways called as the called as a germ line gene therapy, where you correct the defective gene or introduce your normal gene into the germ cells of the body, so that not only you are cured, but your offspring also cured. But, since the gene therapeutic technique that we have developed still are not very efficient and they are also many ethical issues that are involved so far, no germ line gene therapy trails have been permitted or have undergone anywhere in the world. So,

germ line gene therapy involves alteration of the D N A of a gamete or a fertilized egg, changes are heritable passed down from treated individual to the offspring, but currently no germ line gene therapy is being done in humans, so all the gene therapy that we go on talk about today, all confined to the somatic cells of humans, somatic gene therapy is what is going to be focus of today's discussion.

(Refer Slide Time: 21:54)



There also two different kinds of genetic disorders when we talk about gene therapy. There are genetic disorders which hare caused by single gene defects, there are also genetic disorders what is called as multiple genes, so this are called as monogenic versus polygenic diseases, so because if a monogenic disease, a single locus is defective and therefore, is responsible for the disease, some of the examples are; sickle cell anemia, for example, that beta goblin gene defective in sickle cell anemia, hypercholesterolemia where they are the very high levels of cholesterol, because they do not have receptor gene coding for the low densitive lipoprotein receptor, L D L receptor is not present in this gene, therefore L D L cannot be effect taken up by the pair cells. Therefore L D L levels becomes very high in the circulating blood, therefore they develop etios fibrosis and all kinds of problems, cystic fibrosis again it is a monogenic disease, because the gene involved is called C S T R cystic fibrosis trans membrane regulator.

Hemophilia, the defective gene is either factor eight or factors nine, which are responsible for blood clotting, scid we just now discussed the gene involved is

Adenosine deaminase. So, these are all examples of what are called as monogenic disorders where the defect disease is because of a defect in a single gene, but there are also very complicated disorders which are called as polygenic traits, they depend upon multiple environmental factors as well as life style, they are much more complicated diseases like heart disease, cancer, diabetes, alcoholism, schizophrenia these are all called as polygenic traits, and it is not because of the single gene that you get in disorders, complex interaction between the genetic complement and the environment and as well as your life style can lead to this kinds of disorders, and we are still not equipped to treat any of this polygenic disorders. So all the discussion about human gene therapy is been focused on, somatic gene therapy as well as monogenic disorders, these are the two major areas of gene therapy that is currently going on in the case of the human gene therapy.

(Refer Slide Time: 23:53)

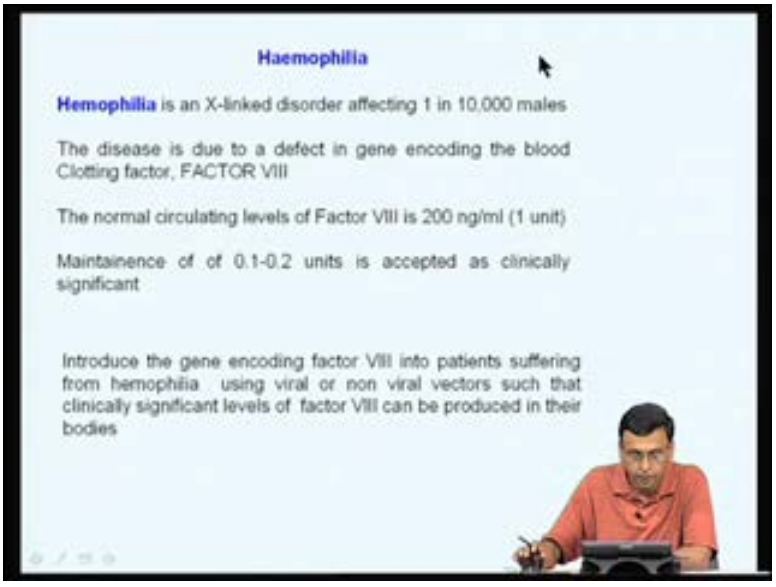
So, many of some of the examples have given here, what kind of monogenic disorders are being treated or by or being aimed to be cured by gene therapy, was still are not cured any anywhere also in chemical trails are being aimed to the cure by gene therapy, things like scid A D A deficiency, alpha 1 antitrypsin deficiency, this leads to disease called emphysema, chronic granulomatous disease, cystic fibrosis, familial hypercholesterolemia, fanconi anemia, gaucher disease, hunter syndrome, parkinson's hemophilia these are all some of the disease which were all examples of monogenetic

disorders where the defect is single gene, therefore if you can introduce that correct gene by a viral or a non-viral vector, can you cure this disease this is what is being aimed at.

(Refer Slide Time: 24:39)

So, I just give two three examples of gene therapy for genetic disorders just to get an idea, there are number of disorders at the end of this lecture I give a series of references, one can always go through this references to get yourself updated on, various other kinds of genetic disorders.

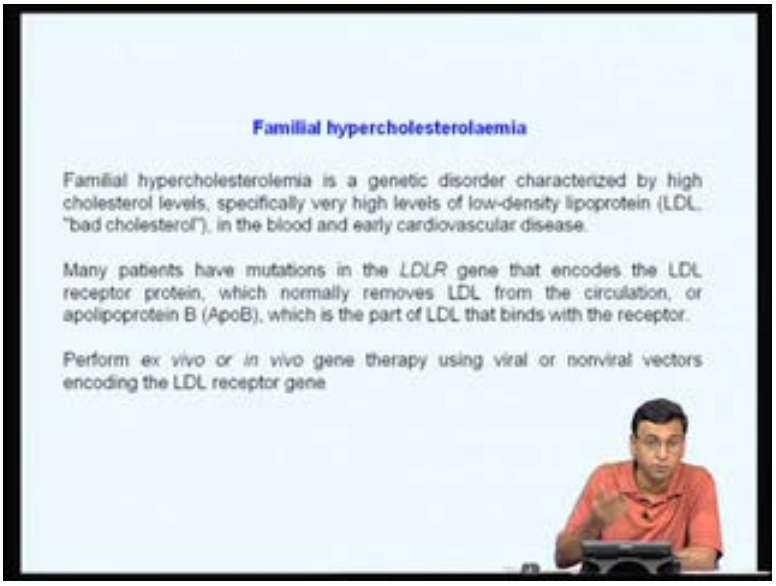
(Refer Slide Time: 24:56)



Hemophilia is one of a very attractive kind gene therapy. The hemophilia is an x linked disorder affecting one in ten thousand males, the disease is actually due to defect in gene encoding for a blood clotting factor called factor eight, that is also what is called as a hemophilia b, where the defect is because of a defect in gene coding for factor nine another clotting factor. Normally, in all the normal individuals, the circulating levels of factor eight is about two hundred nanograms per m l, the factor eight is produced from the liver and suck in to the blood, so all of us are normal, because whenever you have a cut the blood immediately clots, that is because the factor eight is we are producing factor eight, and we have about two hundred nanogram per m l in your blood of the factor eight protein, but even if you maintain point 1 to point 2 units, that is 1 tenth or 1 twentieth of this levels, you can still your blood can still clot and it is still considered clinically significant.

So what is you are being aimed at gene therapy for hemophilia is, can you use a non-viral vector or a viral vector to introduce the gene coding for factor eight in to these hemophilia individuals, so that they can maintain about point 1 to point 2 units which is one tenth or one twentieth normal levels of factor eight, unfortunately even this we have not been able to successfully achieve till date, because number of problems associated with gene therapy is still. So, the strategy has been introduce the gene encoding factor eight into patients suffering from hemophilia, using viral or non-viral vectors such that clinically significant levels of factor eight can be produced in their bodies. This is what is being aimed for hemophilia for gene therapy.

(Refer Slide Time: 26:30)

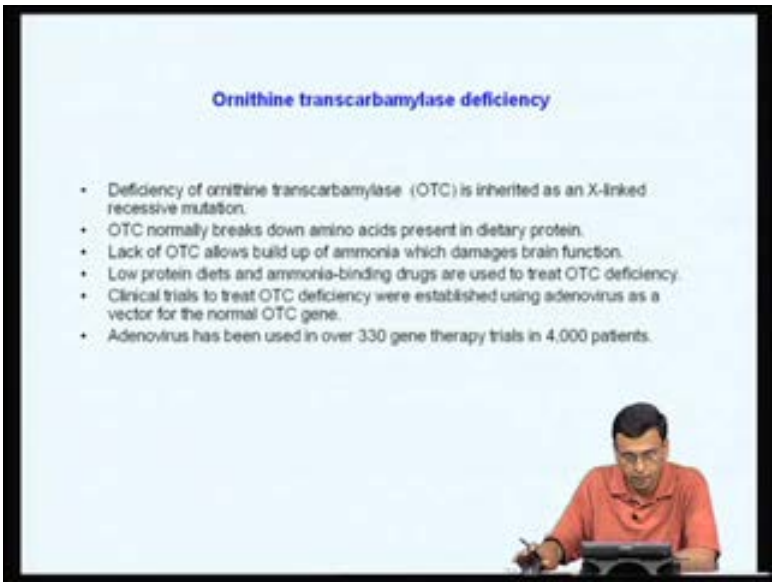


Similarly, if you take a disease like familial hypercholesterolemia, it is a genetic disorder characterized by high cholesterol levels, especially very high levels of low density lipoprotein, which is called as the bad cholesterol in the blood as well as, I mean in the blood leading to early cardiovascular diseases. Many patients suffering from familial hypercholesterolemia have mutations in the L D L R gene which codes for the low density lipoprotein receptor protein, which normally removes L D L from the circulation. They may also have mutation another gene called as apolipo protein b which is the part of the L D L that binds with the receptor, so you have mutations in either of these 2 genes it can be manifest in the form of a familial hypercholesterolemia. They have very high levels of cholesterol in the blood, because the L D L is not taken inside the liver, because the liver size that do not express that L D L receptor gene.

So, what is normally done in such cases, what is being aimed you see, can you now target using a non-viral or a viral vector can you target L D L receptor gene into the liver cells of this patients, so that liver cells now express L D L receptor, therefore, L D L can be taken inside the liver, and the levels of L D L in the blood can be lower, this is what has been aimed at, perform ex vivo or in vivo gene therapy using viral or non-viral vectors encoding the L D L receptor genes. So, take hepatitis from these individuals, culture this hepatitis with you, allow them to prolifate, transfect them either retroviral vectors or adenoviral vectors or non-viral vectors, and once the gene got transfected, and take this hepatocytes back. And infuse them back through the portal into these animals,

so liver cells go and repopulate the livers, and now the liver starts expressing L D L receptor gene and the L D L can be now cleared from the circulation, or using again non-viral or viral vectors directly introduce the gene in to the liver, so that the using in vivo gene therapy, so that the liver cells now starts expressing L D L receptor. This is what has being aimed at.

(Refer Slide Time: 28:22)



Similarly, another important disease called ornithine transcarbamyalse deficiency O T C, now deficiency of ornithine transcarbamyalse O T C is inherited as an x linked recessive mutation, the job of O T C is it normally breaks down amino acids that present in the dietary protein, if you do not have O T C, it leads to build up of ammonia which damages brain function. So they going to coma, low protein diets and ammonia binding drugs are used to treat o t c deficiency, clinical trials to treat o t c deficiency were established, using adenovirus as a vector for the normal o t c gene. Now, you code take the o t c gene, put it in a adenoviral vector, and you can take this recombinant retroviruses and directly inject them into this o t c patients, so that they now started expressing enzyme and patients can be cured off this diseases. These are couple of examples of genetic disorders being aimed for cured by gene therapy, the number of acquired disorders are also are targets for gene therapy.

(Refer Slide Time: 29:25)

For example, gene therapy for the treatment of cardiovascular disorders, revascularization of blocked coronary arteries leading to myocardial infarction, you know the coronary arteries when they get block, it results in the myocardial infarction or heart attacks. So, what is being aimed by gene therapy is that, can you introduce vectors non-viral or viral vectors encoding angiogenic factors, like vascular endothelial growth factor or fibroblast growth factor V E G F or F G F that promote growth of new arteries, leading to revascularization of blocked arteries at the site of ischaemia. So, new blood vessels can grow around the infect, therefore the heart will now get new blood supply, can you now aim at it, so can you now introduce vectors either adeno viral or retro viral vector to the site of this expedia, and promote the growth of new blood vessels by expressing genes encoding vascular endothelial growth factors or fiber blast growth factor, this is what being informed gene therapy for treatment of cardiovascular disorders. Similarly, local expression of anti-thrombotic genes, for example gene therapy aims at dissolving blood clots following angioplasty, by introducing vectors encoding cyclooxygenase 1, which is involved in the synthase of prostacyclin which is an anti-thrombotic agents. So, these are some of the examples of treating acquired disorders, these are not genetic these are acquired disorders using gene therapy.

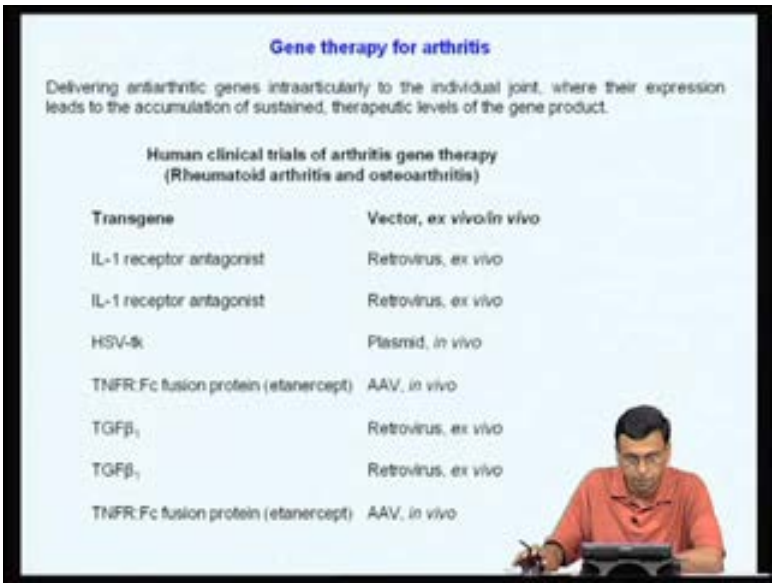
(Refer Slide Time: 30:48)

Again gene therapy for the treatment of cancer, again a very one of the early successes eh or early human trial gene therapy performing the area of cancer, where in treatment of metastatic melanoma by adopting immunotherapy using what is called as tumor infiltrating lymphocytes with vectors encoding interleukin 2. Now, we have our immune system and normally the lymphocytes should recognize this tumor cells, because this tumor cells express many foreign proteins or very high levels of proteins, which are not normally express in the normal cells, therefore these lymphocytes somehow should recognize this tumor as foreign and destroy, but for some reason these 2 lymphocytes are unable to recognize this tumors as foreign, although there are lymphocytes in the vicinity of the tumor cells, therefore there are called as tumor inflating lymphocytes.

So, what you do is you isolate this tumor infiltrating lymphocytes, and then introduce by viral or non-viral vectors genes encoding cytokines like interleukin 2, and these lymphocytes expressing high levels of interleukin 2 if you now inject the back to the body, they now and then go out of the tumor and now express very high levels of interleukin 2, and therefore, attractive cells were using of tumor, and now the t cells will go and then recognize the anti-genus being express on to tumor cells and destroy the tumor cells. This is one approach, another approach is to use viral or non-viral vectors encoding tumor suppressor gene, cytokines, alloantigens and so on and so forth. In fact more than half of the clinical trials, even more than that of the human trials that are going

that of your gene therapy is same at various types of cancers. So, a large number of human trials are going on aimed at treatment of cancer using gene therapy

(Refer Slide Time: 32:34)



Gene therapy for arthritis, delivering anti-arthritic genes intra-articularly into the individual joint, where there expression leads to accumulation of sustained therapeutic levels of the gene products, now what kind of genes we are trying to introduce for treatment of arthritis, I have done a list here, you can introduce for example, I L-1 receptor antagonist or H S V thermo kinesis, tumor necrosis factor receptor, F C fusion commercially known as etanercept, using vectors like adeno associated virus, here people are trying to introduce the genes using retro virus vectors transforming that factor beta and so on and so forth. So, using either retro viruses or direct plasmid non-viral vectors involving plasmid D N A or adeno virus adeno associated virus as vectors, people are trying to introduce a number of anti-arthritic genes directly into the joints to see localized expression of these anti-arthritic genes, can they have a therapeutic effect leading to the cure of arthritis.

(Refer Slide Time: 33:35)

Now, only cases there are many such examples I will not go to all the details, I can always go and look up into all kinds of disease that are being aimed for gene therapies. The three major challenges in all these whether it is for a genetic disorder or acquired disorder, the three major challenges in gene therapy are delivery of the genes, sustained expression of the delivered gene and regulation. These are the three major issues for gene therapy, whether it is a genetic disorder or acquired disorder, the major issue that being addressed is can you now deliver the gene to as many cells in the body as possible, efficiency of delivery is very important, and once you deliver the gene, can the delivered gene explained in a sustained manner.

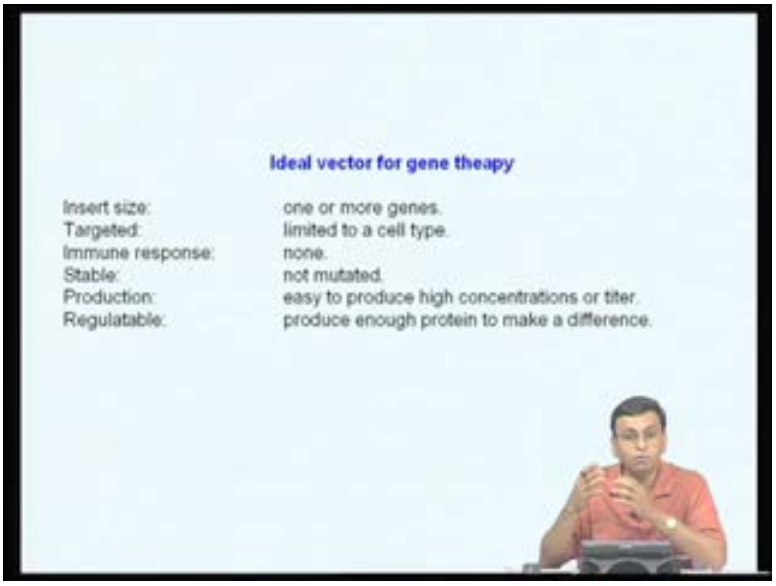
The major problem that you have with all the viral and non-viral vectors that are we have discussed in the last few classes, that are being used for gene therapy is that they all express, whether the whole idea of gene therapy is to for the rest of his life, he does not require protein therapy, that is what is the ultimate aim of gene therapy, that means once you perform a gene therapy and individual, the protein should be made for his life time. Even so far we have not achieved that kind of a results, but even we introduce gene can expresses for say few months or few years it is a major breakthrough, because protein therapy you have to take at a much frequent intervals, so they introduce genes should be able to express for sustain periods, so that have a long term advantage, but for the 1 for the major problems in gene therapy is that, whenever you introduce these genes using

either retro viral vectors or non-viral vectors or other adeno viral vectors so on and so forth.

The expression is there for initial a few weeks or few months and after sometime the expression drops down, and they say this is 1 of the major problem, lack of sustained expression is 1 of the major problems why gene therapy has not been still a clinical reality, again regulation, many of the vectors that we are using use constitutive promoters like this virus promoters and so on and so forth. So, introduce gene is constitutively expressed, but ideally you would like to express the introduced gene also to regulate the same way as the endogenous gene, it should have its own native promoter and so on and so forth, so the regulated expression of the introduced gene is another dream of gene therapists all over the world, which we still not achieved.

So the major steps in performing gene therapy is that you need to package the gene that means you need to put in the right vector system either viral or non-viral, you have to protect the gene, you have to make sure that when you introduce the gene into the body it should not get destroyed either by the immune system or by nucleases and so on and so forth. It is the non-viral vector, and then we deliver the gene to the nucleus and express it over a sustain a period of time, these are three major objectives of gene therapy. So, to achieve this one, what is the most important criteria or what is the most important step in performing gene therapy is, to develop what are called as safe and efficient vectors, whether is a viral or non-viral you need to have safe and efficient vectors to achieve all these 3 major challenges that are required for successful gene therapy.

(Refer Slide Time: 36:54)



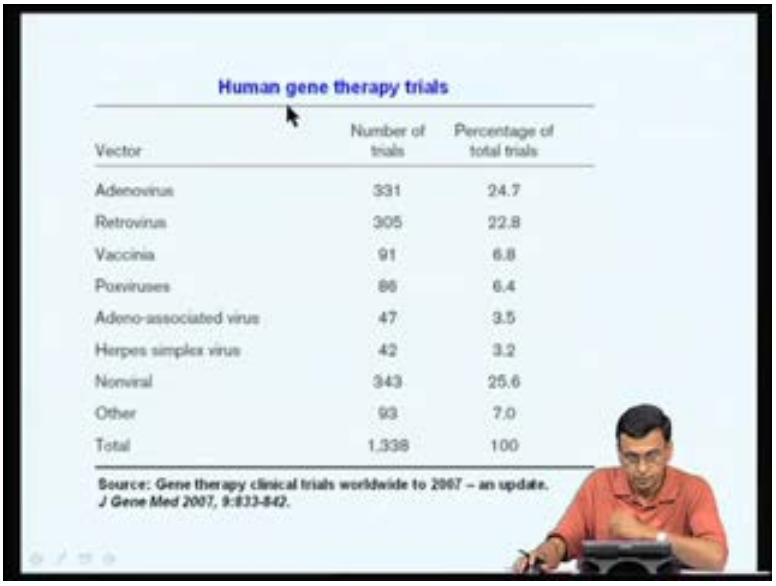
So, what is an ideal vector for gene therapy, an ideal vector would be, it should be carry it should be able to carry one or more genes of your choice, it should be targeted that means, for example if you want to treat a familial hypercholesterolemia labia, your gene should be expressed in the lever not other tissues. So your vector system should be designed in such a way that it only target to the lever, similarly if you want to treat a disease like cystic fibrosis, your vector system should be in such a way that will only go and target to lung tissue, but there are also disease like hemophilia where your objective is to express factor eight or factor nine in the blood, so in such case it does not matter, you do not require a targeted vector, does not matter whether the vector is expressing your gene in muscle or lever or lung, as long as the factor eight is released from the blood, and your able to maintain clinically significant levels of factor eight you have achieved your objective. So, there are certain genetic disorders where targeting is not required, but there are certain disorders where targeting is efficient, for example Parkinson disease, you have to introduce the gene going for tyrosine hydroxylase right here into the substantial nigra in the brain, only then it will have decide effect you cannot reduce a lever or anywhere else.

Similarly, arthritis you have to target the gene or the vector into the joints, only then you will have clinically beneficial. So, target a delivery and expression of the vector is very important, the vector should not induce any immune response. One of the major problems with some of the vector systems both non-viral as well as especially adeno

viral vector systems is that when you introduce suppose the expression level goes down after say three or four months, then again you are inject the vector, somehow the vector level encoded proteins which are present on the vector they would induced immune response in there first time. So, when induced second time our immune system will recognize that is a foreign and the vector will be destroyed, so even before the vector gets into the target tissue they will be eliminated by your immune system, so one of the major objectives of gene therapy.

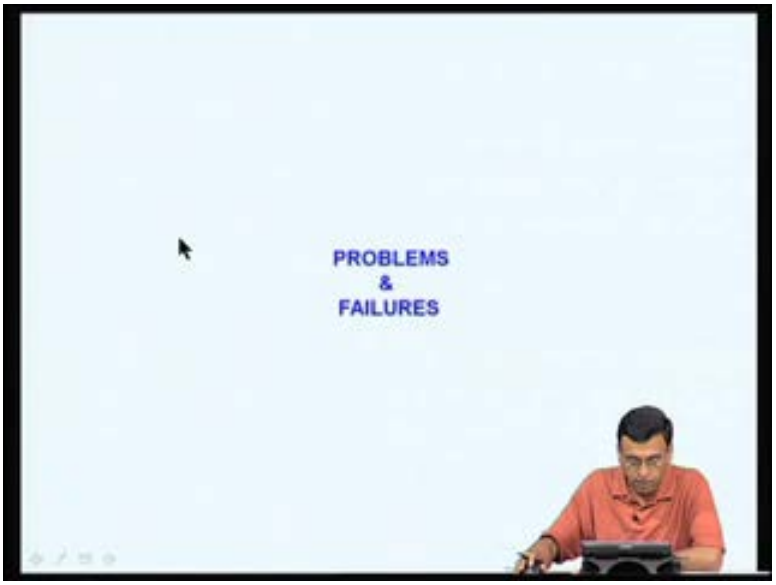
So, develop vectors which are not immunogenic, so it should not the vector should not illustrate immune response from our body; it should be stable, it should not under any mutations, it should be easy to produce especially if you talking about viral vectors, you should be able to produce high concentrations of this viruses using packaging or producer cell lines, because cost is one of the major factors in gene therapy regulatable, as I said you should produce enough protein to make a difference, and ideally you should also be regulate the same way as the endogenous gene. So, some of the major characteristics of the ideal vectors, unfortunately either the non-viral or the viral vector system, I would discuss in the last two classes none of them actually fulfill all the criterions of an ideal vector, so we do not have a vector system which fulfills all the criterions of a ideal vectors, so that is the problems.

(Refer Slide Time: 39:49)



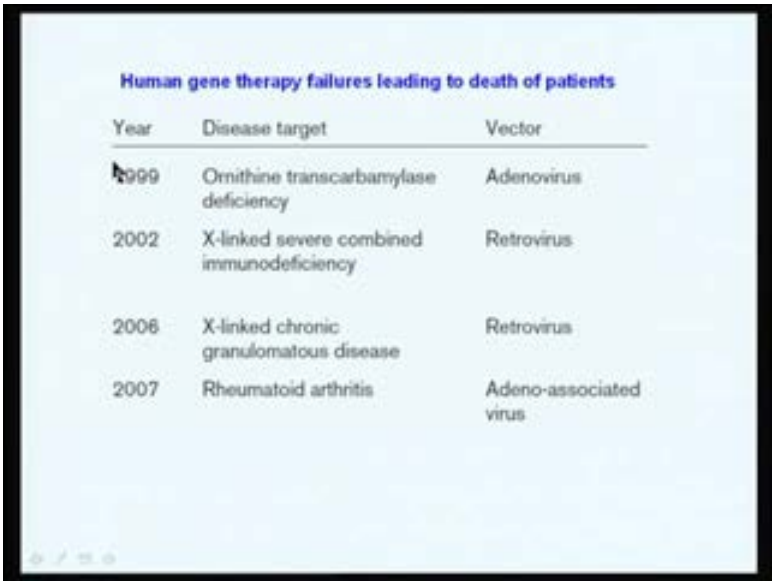
So, despite all these deficiencies and still drawbacks, a number of human gene trial gene therapy trials are going across the world, in fact this is a data took all for 1 of the general published in 2007, and you can see the number of trials are going on across the world using a various vectors systems; adeno virus, retro virus, vaccinia pox viruses, non-viral so on and so forth, a number of trials are going on, as you can see majority of the trials involve the use of a n adeno viral vectors or retro viral vectors and non-viral vectors, so these are 3 most popular vector that are being used for a number of gene therapy trials adeno viral vectors, retro viral vectors and non-viral vectors.

(Refer Slide Time: 40:35)



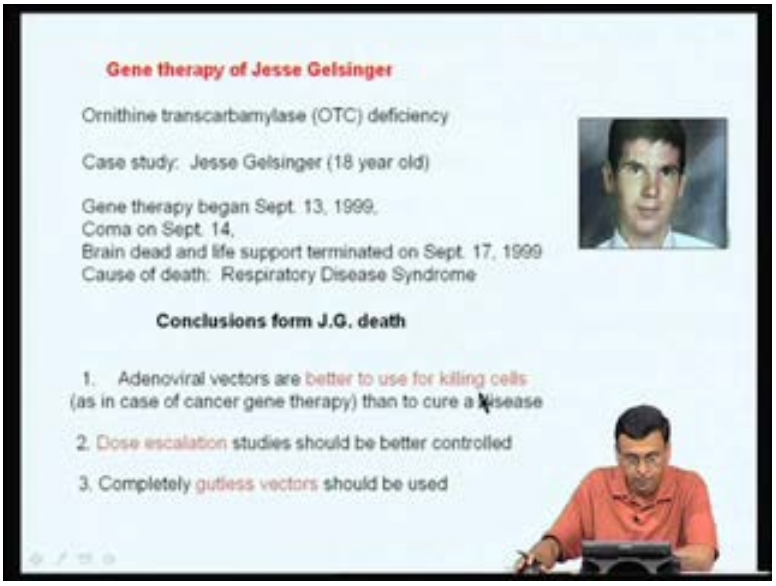
Now, so having disused what is the general strategy for gene therapy and what kind of disease are being cured at, and how to introduce the gene where it is in y o in c 2 or x v o and so on and so forth, as I said the first human gene therapy trial was done first 1990, so it is now two decades, what has happened now, have you regularly achieved the ultimate objective, has human gene therapy as clinical reality, the human gene therapy is still not a clinical reality, because they are still under various stages of clinical trials, still it has not reached the stage where you can go to a clinic and ask the doctor to perform gene therapy for either genetic acquired disorder, why is the reason, what is the problem, what are the problems associated with all this two decades of human gene therapy research. Let us now look at 2 or 3 or such major problems which hindered or which hampered gene therapy becoming a clinical reality, I listed four major issues which were the major challenges or major problems or major failures of human gene therapy.

(Refer Slide Time: 41:40)



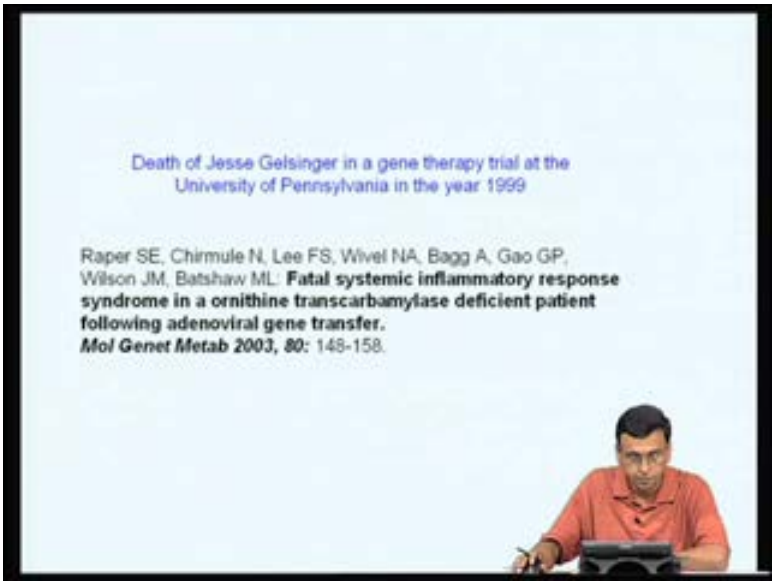
In the year 1999 when a adeno viral vector was actually used to treat a human patient, to cure ornithine transcarbamylase deficiency which we discuss just few slides back, this patient actually died when he was undergoing gene therapy. Similarly, in the year 2002 in France some human patients who were actually treated by gene therapy for scid severe combine immunity syndrome, some of them developed cancer, leukemia where the retro virus was actually used for transferring your gene, similarly in 2006 a woman who was suited for x linked chronic granulomatous disease using retro viral vector also died on 2007, a patient who received a rheumatoid arthritis undergone treatment for rheumatoid arthritis using adeno associated virus into anti-arthritic gene also died at the time during the gene therapy trial. So, from 1990 and 2000 there have been incidents of patients who received the gene therapy treatments, if not all or some of them actually died or developed lot of problems, so I just spend some time to discuss what this problems are.

(Refer Slide Time: 42:53)



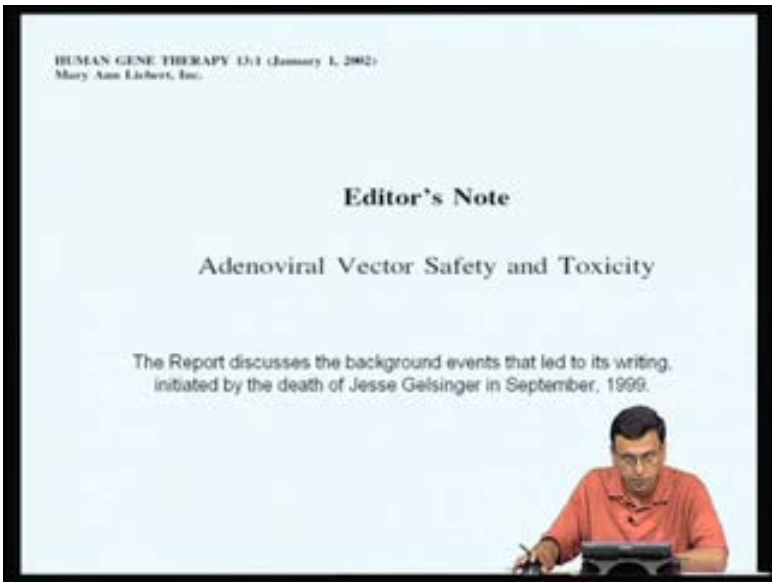
For example, in the 1999 this gentlemen known as Jesse Gelsinger, who was suffering for ornithine transcarbamylase deficiency, was taken in for a human gene therapy trail, where in adeno viruses expressing the O T C gene was introduced into the body, the gene therapy began on September 13 1999, but Gelsinger went into coma on September 14 and he never recovered, he died and the cause was identified as respiratory disease syndrome. Now, a lot of investigations went on to see why did he die, what was the problem and it turns out, it is the problem it is the vector, adeno viruses when you use in high titers can cause a problem, so adeno virus vectors it is decided that adeno virus vectors are better use for killing cells, especially for cancer gene therapy then for a genetic disorder, and dose escalation studies should be better controlled, you cannot inject very high dosage of this adeno viral vectors, and you need to use adeno viral vectors which are non-immune genic which are less immune genic. These were the conclusions.

(Refer Slide Time: 44:02)



In fact the death of Jesse Gelsinger in gene therapy trial in the year 1999 was one of the most highly investigated gene therapy trial, failures and a number of papers have been published to understand what exactly, was the cause for the death, it is published in every respected journals.

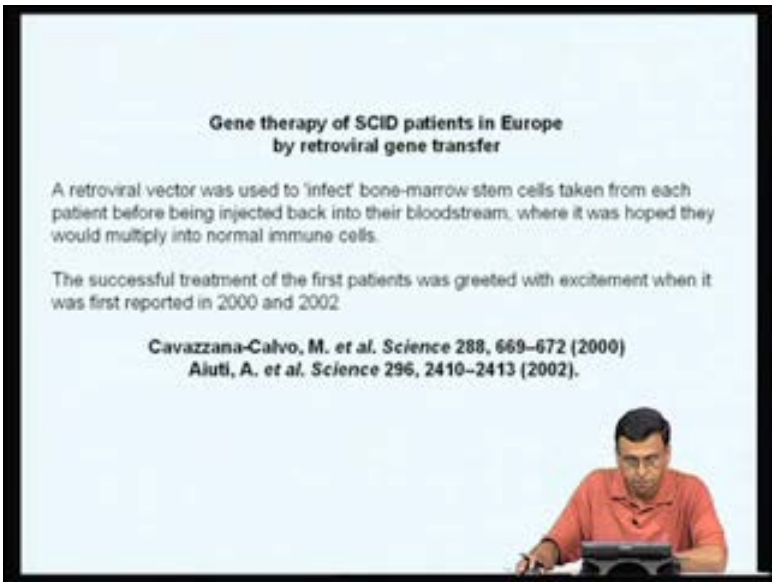
(Refer Slide Time: 44:21)



In fact there is a very nice article written by the editor of human gene therapy which discusses the background events that led to the death of the Jesse Gelsinger in September 1999. So, once this kind of a problem maintain, a lot of people went into understand

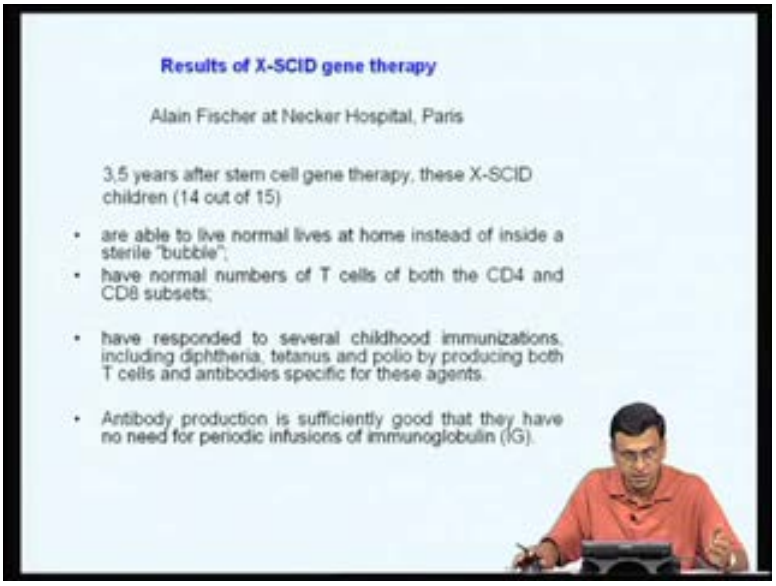
what was the problem and why this failure happened, so that this kinds of trials or briefly stopped. But once conclusions are drawn, you make sure that you do not repeat some of this mistakes and you resume the gene therapy trails.

(Refer Slide Time: 44:51)



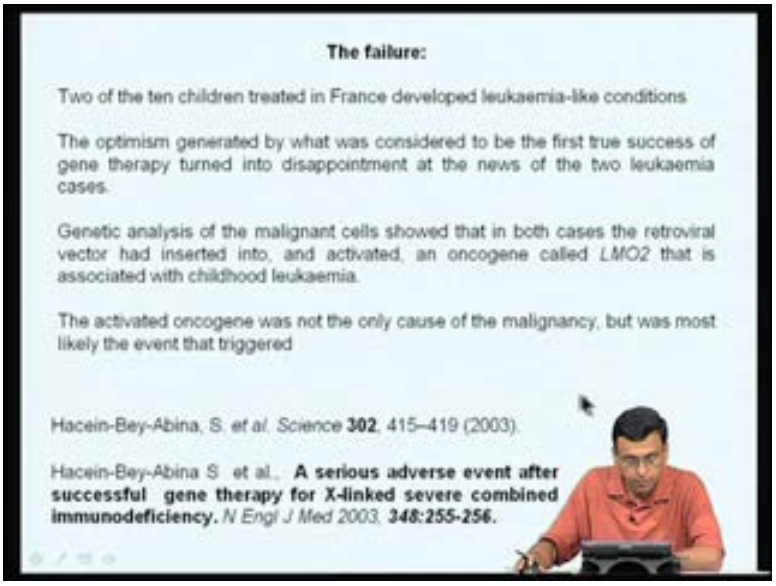
The other important drawback that happened was, in Europe, especially in France one about twelve to fourteen patients were actually treated for scid severe combined immune deficiency syndrome, again using retro viruses for gene transfer the successful treatment of these patients was greeted with excitement and was published science, a reputed journal in the year 2000, 2002 saying that they had a clinical benefited results.

(Refer Slide Time: 45:19)



And I can See here three to five years after the performing the gene therapy for the scid of fourteen out of fifteen children were able to live normal lives instead living in the sterile bubble, they had normal numbers of t cells of both C D 4 and C D 8 subsets, they responded to several childhood immunizations, that means the immune system is they have the good t cells that is unavailable to vaccinations, including diphtheria tetanus and polio by producing t cells and anti-biotic specific for these antigens indicating that the immune system became normal, the anti-biotic production is sufficiently good that they have no need for periodic infusions of immunoglobulin. So clinically it was very successful, because they had clinically beneficial results, following the gene therapy.

(Refer Slide Time: 46:03)



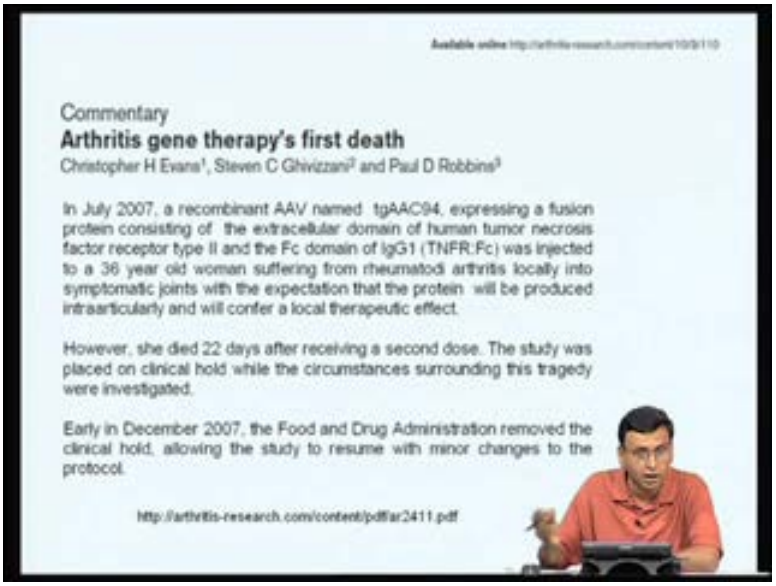
But the problem was 2 of the ten children treated in France developed leukemia like conditions, so the optimism that was generated by what was considered to be the first true success of gene therapy turned into a disappointment at news that 2 out of ten children actually developed leukemia. In fact genetic analysis of the malignant cells actually showed that when they use the retro viral vector for introducing this gene, for treating the scid it turned out this retro viral vector and went and integrated in to this very specific region in the human chromosome, and as a result it activated an ancogene called L M O 2. So in the retro virus went and integrated here, because these retro virus are very power powerful L T R's, it activated this ancogene and when this ancogenes were over expressed it led to leukemia.

So, the culprit turned out to be integration of the integral vector to a very specific region, result in the activation of the ancogene was responsible for development of leukemia in the children who underwent gene therapy. So, the activated gene was not only cause of malignancy, but was also the likely event that triggered leukemia, so again all these were investigated thoroughly, so what is very important about gene therapy is that once they identified the problem, they went on investigating identified what is the cause, why did these children got leukemia and they found out this is the molecular mechanism by which these children developed cancer, and therefore attempts of now we have been to see how you can avoid, and how you can in the future trials.

(Refer Slide Time: 47:43)

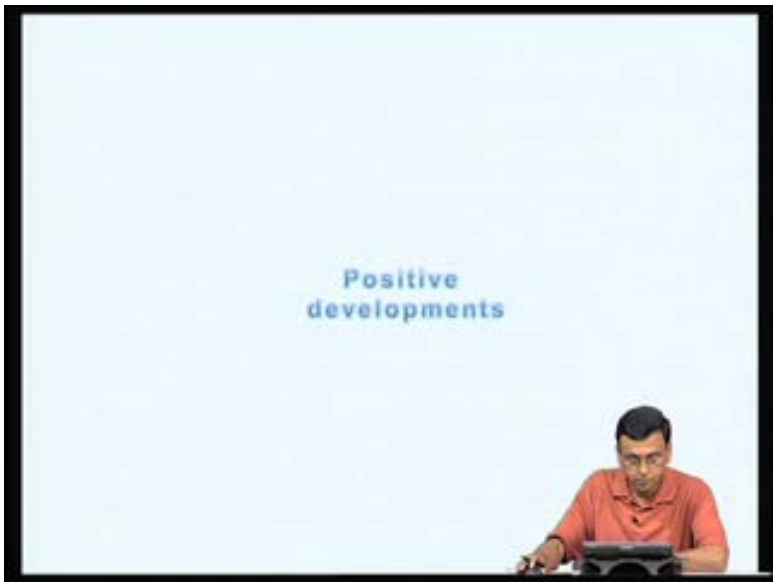
And a few years later another child, a third child also developed cancer, who undergone the same thing so the first trial was stopped in October 2002, and other trials with 3 months later after the two children in the trials down cancer, but on 24 January 2005 the French medical regulatory authority announce that, a child that who was by the same Fischer group in April 2002 also known as cancer. So three out of ten children developed leukemia or cancer indicating that there are some problems with retro virus mediated gene transverses, some more research needs to be done.

(Refer Slide Time: 48:18)



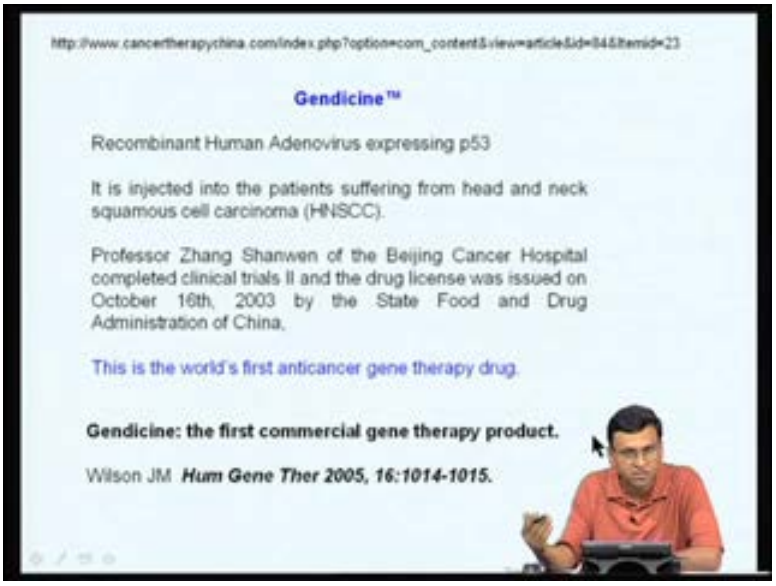
Similarly, in the year 2007 a women who was undergoing treatment for arthritis, she was actually being treated July 2007, using recombinant adeno associated virus expressing a fusion protein consisting of the extracellular domain of human tumor necrosis factor type 2, and F C domain of I g G 1 was injected to a 35 year old women who was suffering from rheumatoid arthritis locally into symptomatic joints with the expectation that the protein which is produced from this adeno associated viral vector will be produced intra particularly, and will have a local therapeutic effect, but unfortunately twenty two days after receiving the second dose this women died, and early in 2007, again this case was investigated and soon it was realized that the cause was not really gene therapy, in fact food and drug admiration removed the halt of the clinical trials and allow this study to resume with minor changes in the protocol. What all these failures actually told is that people went and identified what is the molecule reason, what is the cause for this particular problem and they try to rectify it, and again the future trials these problems were rectified.

(Refer Slide Tim: 49:28)



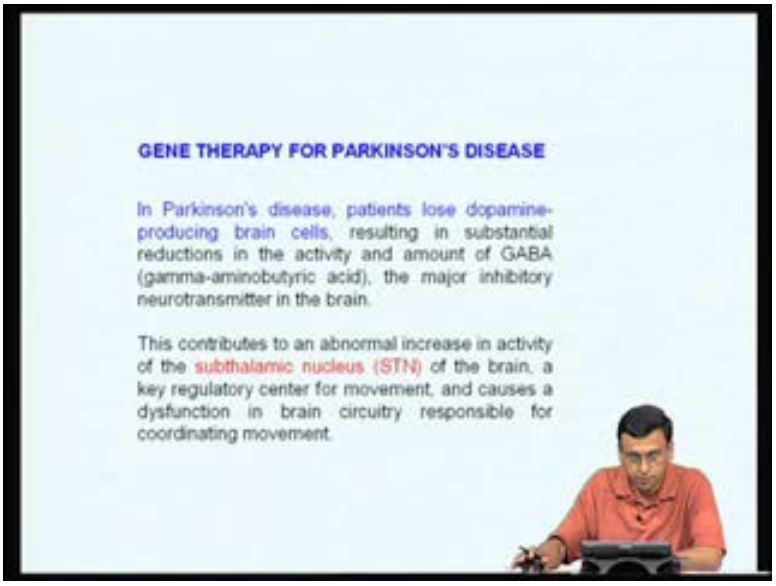
What are the positive developments, so I showed so far that, yes of 1 or 2 very successful human trials, some of them died and some of them had cancer and as a result, the there is only a cautious optimism as for as human gene therapy is concerned, and these are some of the reasons why human gene therapy dispersed 2 decades of research has still not became clinical reality, because we still have not perfected the procedure, what are the positive developments.

(Refer Slide Time: 49:59)



The first worlds anti-cancer gene therapy drug is out in the market this is known gendicine, it is actually recombinant human adeno virus expressing p 53 which is a tumor suppressor protein, and it was injected in to patient suffering from head and neck squamous cell carcinoma, this was done in china hitter by professor Zhang Shanwen of Beijing Cancer Hospital, they completed all the clinical trials, and finally the chinese regulatory authority granted license on October sixteenth 2003, and this is the world first anti-cancer gene therapy drug. In fact it is a very nice article human gene therapy, very prestigious journal in this area about the commercialization of gendicine, the first commercial gene therapy product, so there have been some positive developments to in the area of human gene therapy.

(Refer Slide Time: 50:44)

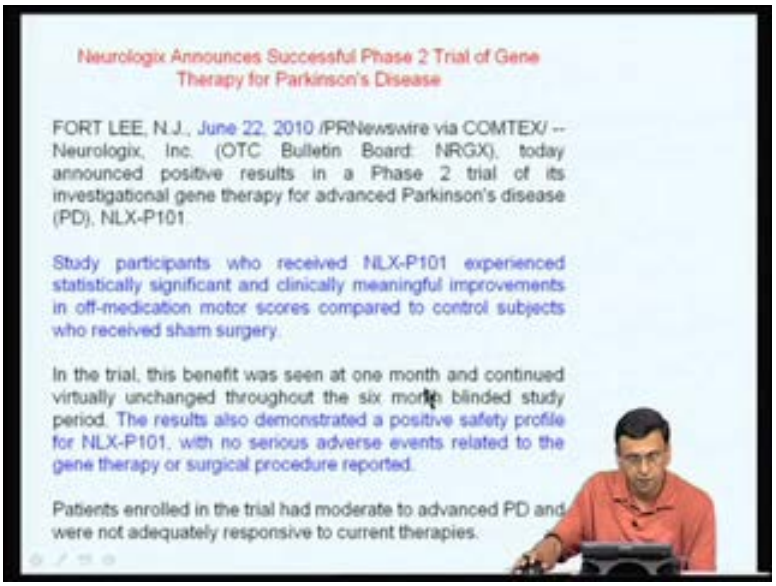


Again, gene therapy for Parkinson disease, now what is Parkinson's disease, in these the patients lose dopamine producing brain cells, resulting in substantial reduction in the activity of the activity and amount of a neuron transmitter called gamma amino butyric acid, a major inhibitory neurotransmitter in brain, this continues the locomotion, locomotary organs of the body, as a result it contributes abnormal increase in the activity of a region in the brain called sub thalamic nucleus, and a key regulatory segment of movement, and causes a dysfunction in brain circuitry responsible coordinating movement. That's why you see these patients; they cannot coordinate their movements anymore.

(Refer Slide Tim: 51:22)

A company called neurologix incorporated is now trying to see can you cure this Parkinson disease by gene therapy, using adeno associated viruses expressing with the gene the interest, and in fact this particular thing if you go to their website, it tells you that they are in phase 2 clinical trials.

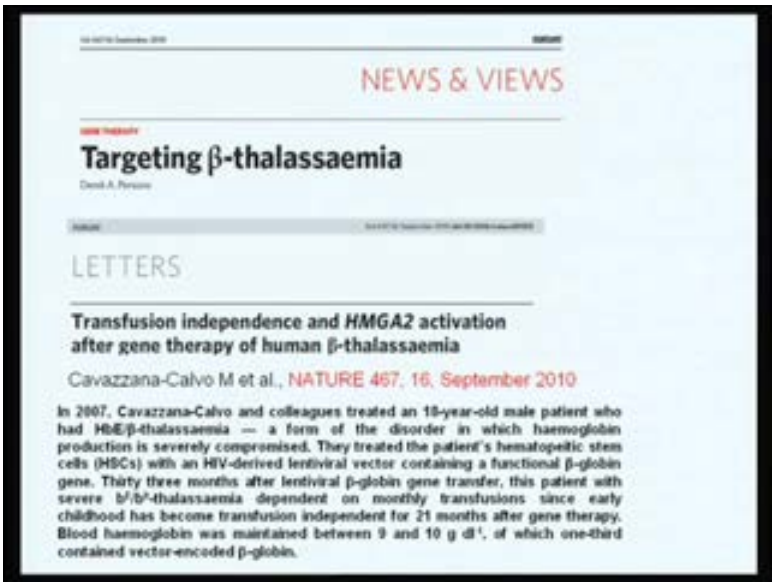
(Refer Slide Time: 51:40)



And this year just June 22, 2010, the company announces very successful phase 2 trial of a gene therapy for parkinson diseases. So the study participants who received this particular A A V vector encoding, the gene of interest for Parkinson disease experienced

statically significant clinical meaningful improvements in off medication motor scores compare to control subjects who received sham surgery. The results also demonstrated a positive safety profile that means, there are no serious advice effects, and it had a clinically beneficial science, so these are all positive development saying that, yes at least for certain diseases gene therapy may become a reality very soon.

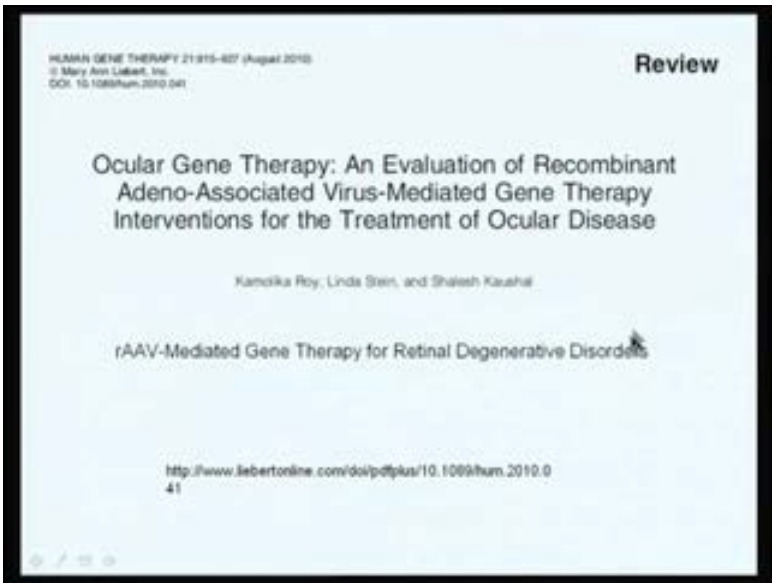
(Refer Slide Time: 52:19)



Today is actually September 23, 2010, last week in nature a very existing development took place, where transfusion independence and H M G A 2 activation after gene therapy of human beta thalassemia. So just last week a paper was published in nature clearly showing that beta thalassemia was successfully cured by gene therapy, and tells you that in this particular case, the gene therapy actually began in the year 2007, so after 3 years of this trial, and what one actually showed in this case, they actually use lenti viral vector for introducing the beta globin gene into this individuals and this patient, who was actually depend on monthly transfusions of beta globin gene. Now, twenty one months after gene therapy he has not received any transfusion, and he is alright and blood hemoglobin was maintained between nine to ten grams per deciliter of which one third contained vector encoded beta globin, that means for a period of twenty one months, the vector which was the lenti virus vector which was expressing beta globin gene is successfully expressing the gene, and he has not required any blood transfusion so far. So, it a very positive developments indicating that yes in this century, there is a good hope that gene therapy will become a clinical reality. There are also number of gene

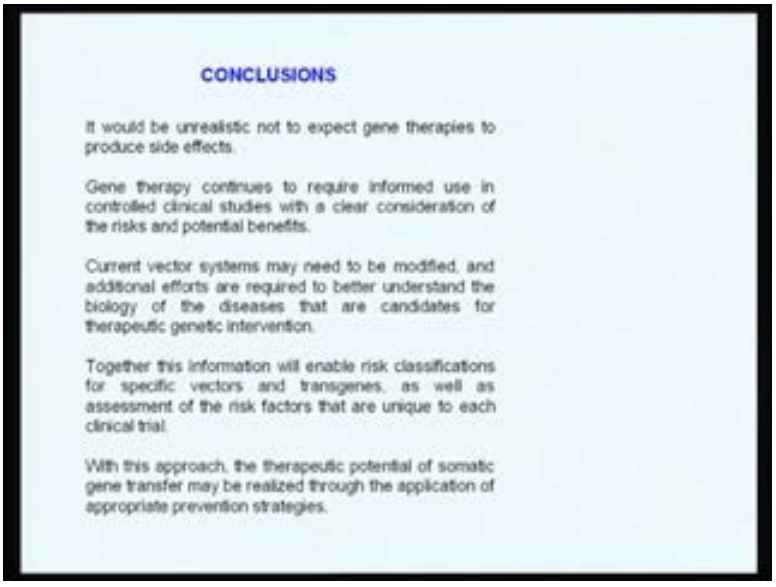
therapy trials going on for correcting a number of retinal disorders, where you use viral or non-viral vector directly inject to the eye, and these are called as ocular gene therapy.

(Refer Slide Time: 53:52)



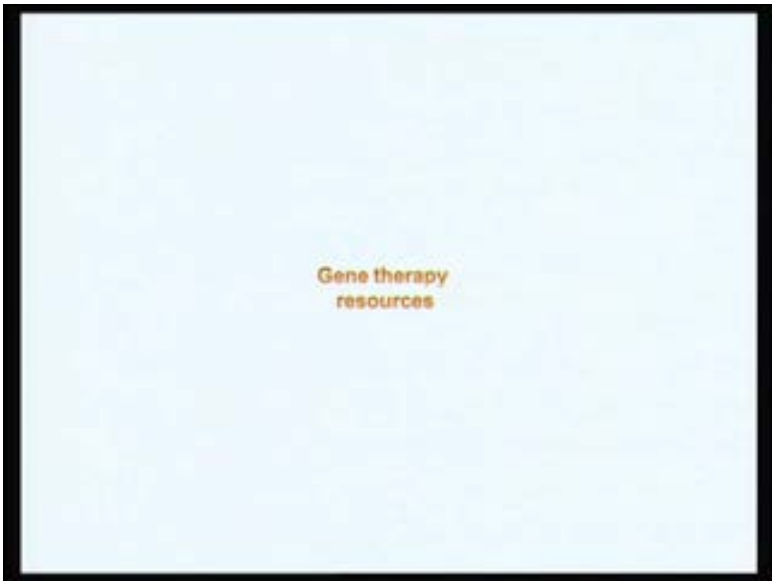
I am not going to the details, because of lack of time, and is very recently review in the last month issue of human gene therapy, on ocular gene therapy an evaluation of recombinant adeno associated virus mediated gene therapy for treatment of a number of ocular diseases. One can go and look up these things.

(Refer Slide Time: 54:09)

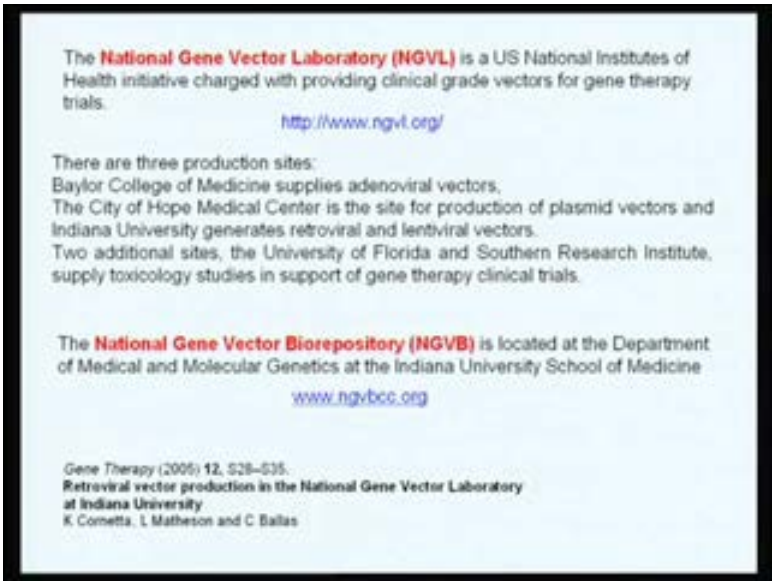


So, what are the conclusions from all that hazards so far, it would be unrealistic not to expect gene therapist to produce side effects, there will be side effects, there may be problems, but gene therapy continues to requires informed use in controlled clinical studies, with a clear consideration of the risks and potential benefits, current vector systems may need to be modified, and additional effects are required to better understand the biology of the diseases which are candidates for gene therapy, together this information will enable risk classifications for specific vectors and transgenes, as well as assessment of risk factors that are unique for each clinical trial, and with this approach the therapeutic potential of somatic gene transfer may be realized through application of appropriate preventive strategies, so I hopeful that we will learn from all this mistakes and some of the success I have told, gene therapy will soon become a clinical reality. There are number of resources for understanding gene therapy, and then gaining more information.

(Refer Slide Time: 55:03)

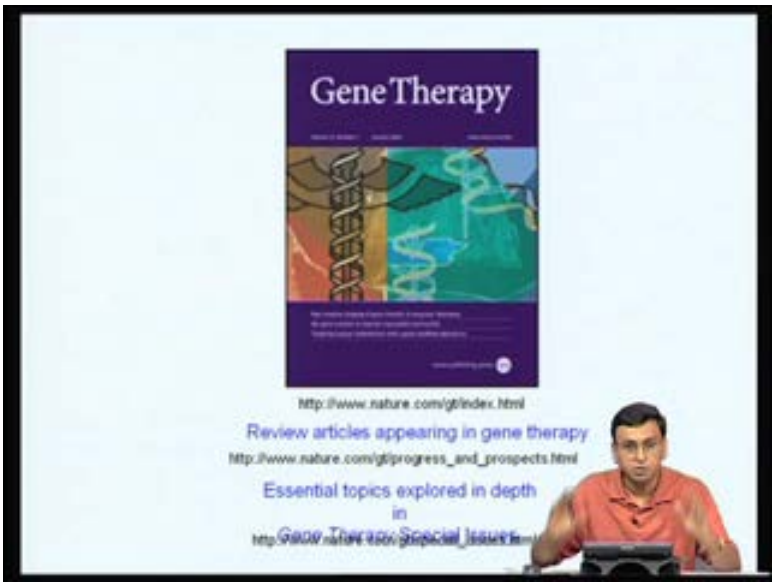


(Refer Slide Time: 55:05)



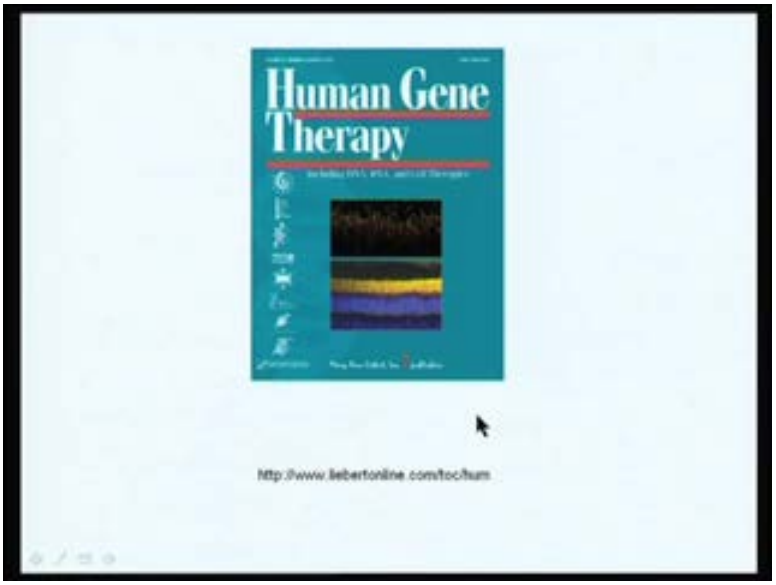
The next few slides I have just listed some of the resources, there is what is called as a national gene vector laboratory in United States, which actually distribute a number of vectors which are being used for gene therapy, one can just write to them and get a number of vectors, they even make some of these vectors and use it in clinical trials, it is also called as a national gene vector biorepository located in Indiana University of Medicine, again assess gene therapy researchers across the globe.

(Refer Slide Time: 55:30)



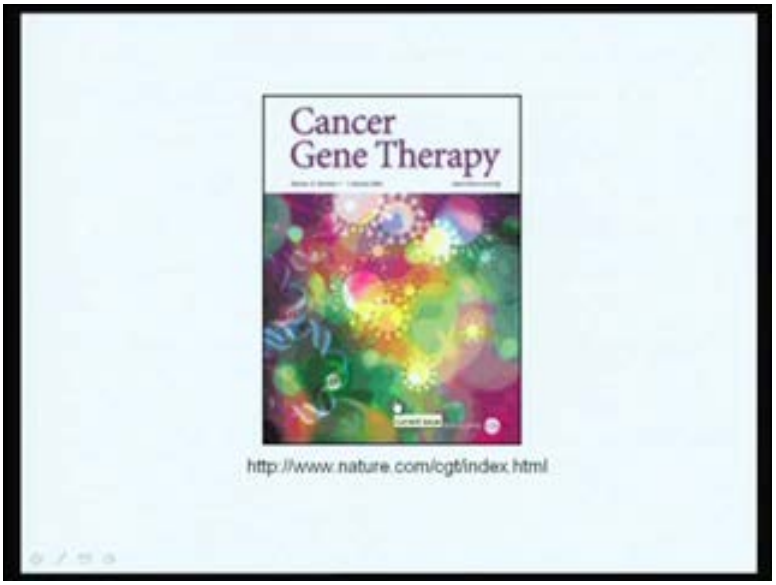
There are very specialized journals, which are now available for publishing gene therapy trials, and gene therapy results, and gene therapy research, there is a journal called gene therapy published by nature group a number of review articles are there if you just go to the website, many of this review articles can be downloaded free.

(Refer Slide Time: 55:48)



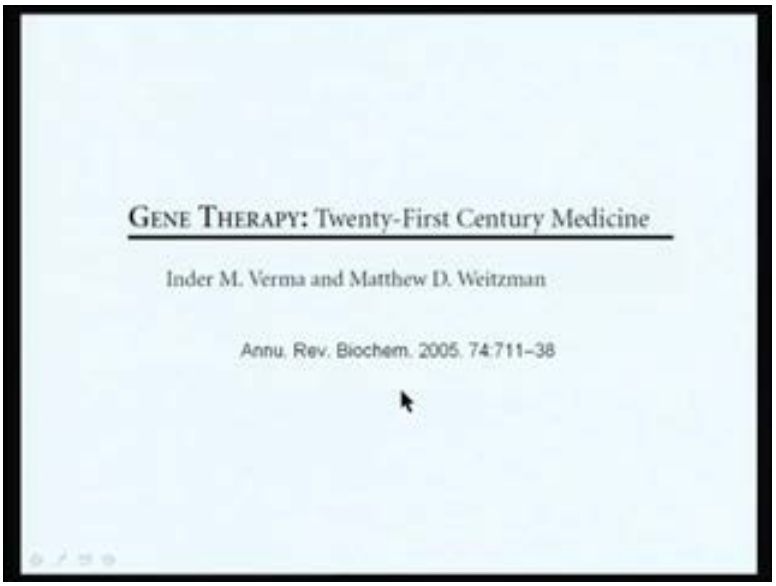
There is another very important called human gene therapy published by Maria and Albert.

(Refer Slide Tim: 55:55)



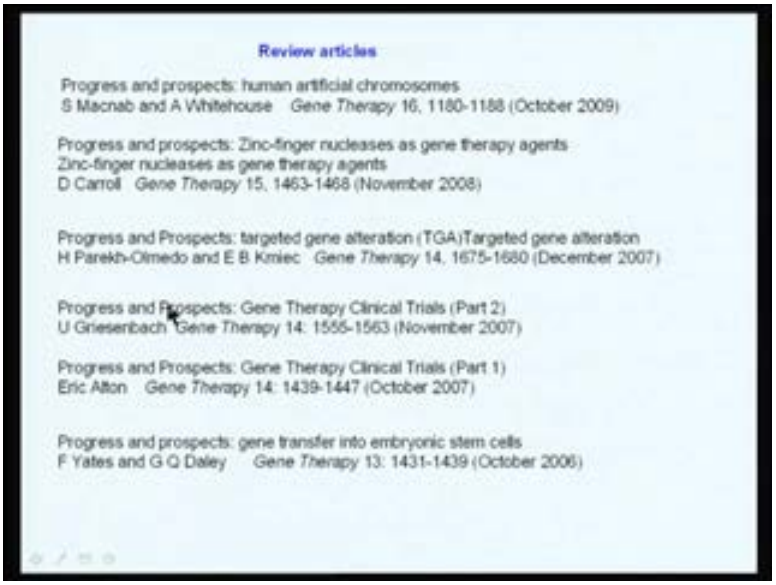
Again a number of interesting articles discuss about the progress being made, there is a very dedicated journal called cancer gene therapy, a number of gene therapy trails going in the area cancer treatment.

(Refer Slide Time: 56:03)



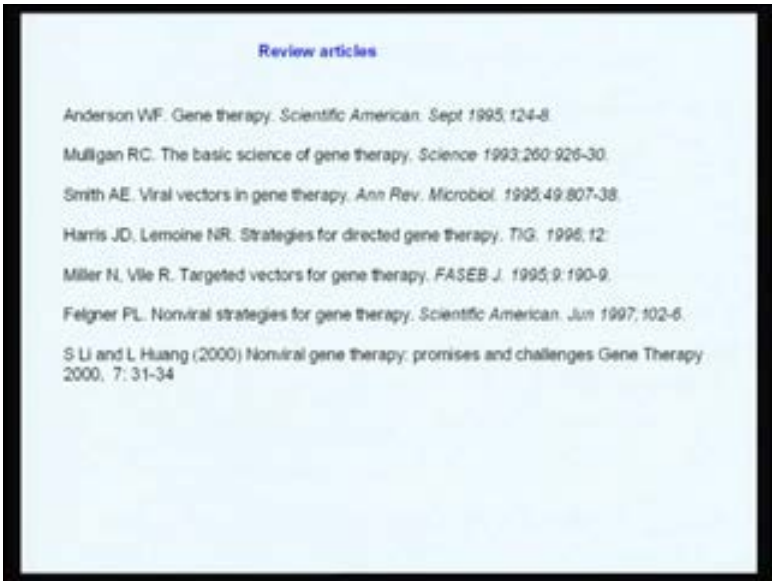
There are beautiful reviews of articles this is for example, appears in the annual review of bio chemistry, about discuss about a various vectors that are being used for gene therapy.

(Refer Slide Time: 56:11)

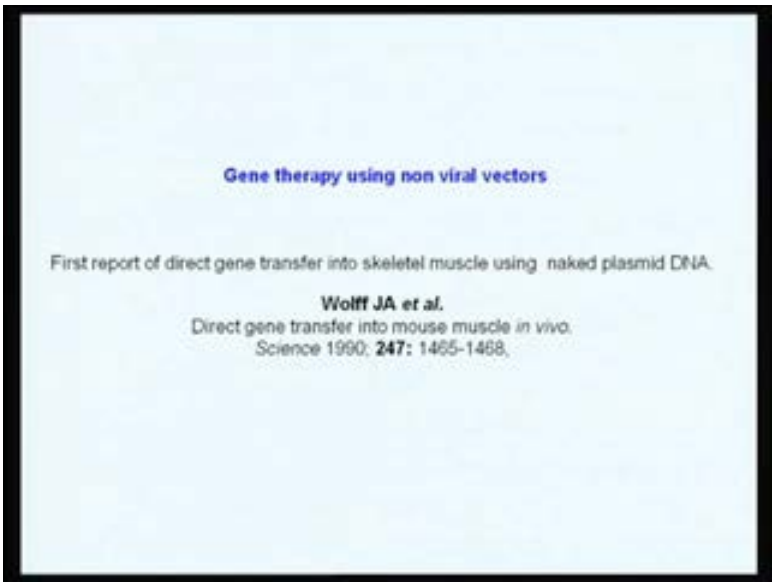


I also listed number of review articles; one can go through all these review articles and enlighten yourself about various gene therapy things. Some, of the very popular reviews written by some of the leading researchers in the field, I listed here gene therapy using non-viral vectors.

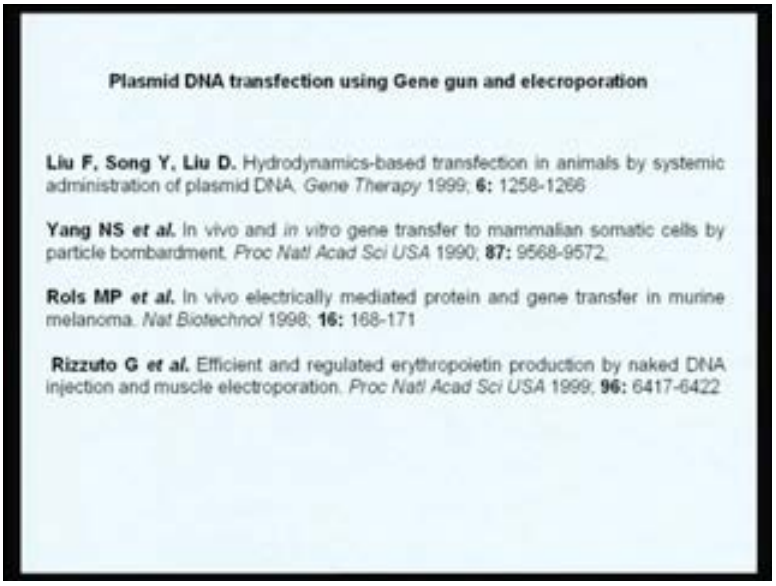
(Refer Slide Time: 56:18)



(Refer Slide Time: 56:27)

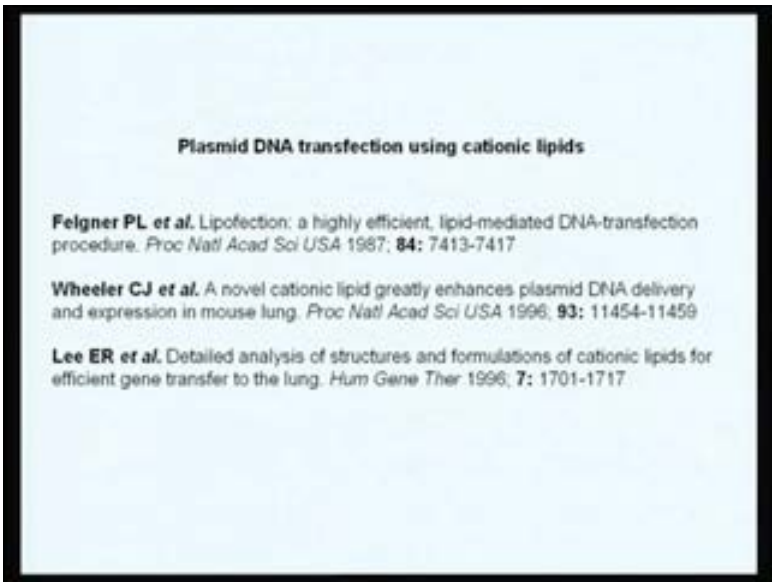


(Refer Slide Time: 56:38)



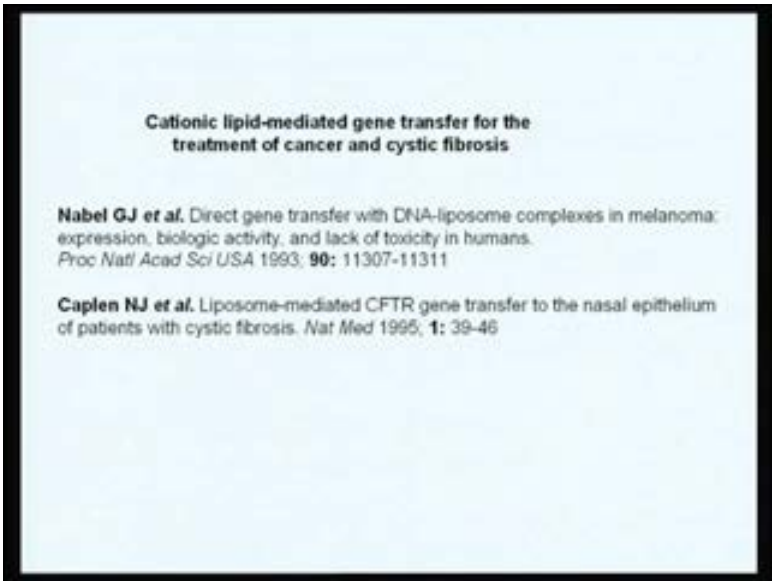
This is the first report of introducing naked D N A into skeletal muscle and demonstrating the expression, this led to using naked D N A for Gene therapy published in science in 1990, and then people started modifying this plasmid gene delivery, using number of reagents for observe.

(Refer Slide Tim: 56:47)



You can improve the efficiency of gene transfer using gene gun, electro oration or using cationic lipids, number of research articles one read, and understand how non-viral gene vectors are used for various purposes.

(Refer Slide Time: 56:55)



For the treatment of cystic fibrosis how cationic lipid mediate gene transfer is being used for treatment of cancer and cystic fibrosis. So, I think I will stop here, I hope that I have convinced you that human gene therapy is a very exiting area, there have been certain drawbacks, there are also been some positive developments, and hopefully some of this will become a reality, and soon gene therapy will become a clinical reality in the next couple of decades, thank you.